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Postmortem Interval Estimation Within the Context of Adipocere Formation

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by

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ABSTRACT

Adipocere is the transformed adipose tissue of a corpse into a wax-like substance. It is known for preserving soft tissue and subsequently obscuring the post-mortem interval (PMI) in a forensic context. The ideal conditions in which adipocere forms on human remains are generally considered to be in warm, moist, alkaline environments. However, these conditions can be highly variable. A searchable database is modeled here as a system for tracking forensic and experimental cases involving adipocere and using those findings to establish minimum PMI estimate for cases of unknown PMI. Test studies are experimentally run to validate the precision of the database in real-world settings. The experiments result in overall accurate PMI estimates. These results indicate that adipocere can contribute to estimating PMI. However, the small sample size of cases with sufficient variables reported, and a lack of standardization in scoring gross adipocere formation must be addressed for future studies.

INDEX WORDS: Adipocere, Decomposition, Time Since Death, PMI

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DEDICATION

This dedication is for my siblings, blood and otherwise, whom I love dearly. Each of you has assisted in your own way, and I sincerely would not have made it through without all of you. Anna and Rose, you are my best friends, confidants, and overall amazing women. I cannot wait to share the next adventures of my life with you both. Sean, you were there for me when I needed you most, and without you I am certain I would not have made it this far. Thank you for constantly pushing me to pursue my dreams, make smart decisions, and never give up. Thank you to my family from the bottom of my heart.

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1 INTRODUCTION

The discipline of forensic science has developed rapidly over the last two decades. Despite this, determining the post-mortem interval (PMI) for a deceased individual is still an inexact science. This is particularly true the longer an individual is deceased, in certain environments such as those that are extremely cold or dry, and when abnormal decomposition processes are present. Such a case as the latter involves the formation of adipocere on the body. Also known as grave wax, adipocere refers to the process of hydrolyzation and hydrogenation that occurs to the fatty acids of adipose tissue after death (Mant and Furbank 1957). Though adipocere has been observed for over three centuries, it is still not entirely understood.

This paper represents a preliminary effort to better establish trends, predictions, and observable limits when it comes to adipocere formation. Given its tendency to preserve underlying soft tissue, adipocere often confounds attempts to determine the post-mortem interval for an adipocerous body. Rather than determining methods for circumventing this obstacle, this thesis asserts that adipocere may be used to help determine PMI. By understanding the ideal conditions under which adipocere forms, the point at which adipocere actively stops forming, methods for determining the stage of adipocere formation, and factors that would facilitate adipocere breakdown it is possible to offset the preserving qualities of adipocere and provide a minimum PMI based on actual experimental and field results.

To demonstrate the diagnostic potential of adipocere in determining PMI, a model is presented in the form of a searchable database that uses existing studies to track applicable variables and compare them to cases of unknown PMI. Such variables include, but are not limited to, place of recovery, temperature, bodily coverings, pH, sex, adipocere descriptors, and chemical composition of the adipocere. An in-depth discussion of the factors involved in adipocere formation and makeup will provide background for the development of the model database and subsequent experimental test runs.

The purpose of such a model is to ultimately provide a tool for practitioners of forensic science. Furthermore, given adipocere's ability to persist on a body and surrounding soil for decades, centuries, and even millennia (Mant and Furbank 1957; Fielder et al. 2009; Papageorgopoulou et al. 2010) it is also relevant to the field of bioarchaeology. This is especially true given adipocere's ability to form on and in bone (Moses 2012). Though not explicitly investigated here, the model and its subsequent discussion is a step towards increasing discourse on the utility of adipocere in both modern and archaic contexts.

2 LITERATURE REVIEW: AN EXTENSIVE SUMMARY OF ADIPOCERE AND ITS PROPERTIES

2.1 History and Description

Though Antoine Fourcroy was the first to define and describe adipocere in 1789 (O'Connor et al. 2011), it was Sir Thomas Brown in the 17th century who appears to have first mentioned a soapy, fatty substance developing on corpses in the literature (Browne 1658, 53; Mant and Furbank 1957; Kumar et al. 2009). The Latin-derived word "adipose" translates to "fat wax" (Mant 1987; Moses 2012). While in its earliest stages adipocere may not be visible macroscopically (Mant and Furbank 1957, 32), as formation progresses it first appears white or grey, "wet and slimy", and resembles a "waxy, cream-like paste in the process of thickening" (Widya et al. 2001, 329). Over time, it will become hard and crumbly (Hanganu et al. 2017). Though adipocere is typically white or grey, it can also appear pink, light green (Hanganu et al. 2017), or yellow (Teo et al. 2014). Under florescent light, adipocere appears white, purple, or violet and this method can be used to identify adipocere on remains (Evans 1963, 60). Adipocere is often recognized by a foul, cheesy, or ammonia-like aroma (Forbes et al. 2005a; Magni et al. 2021), but Evans (1963) notes how difficult and variable it can be to assign an odor to adipocere. However, Mant (1957) gathered from field studies that an ammoniacal odor is especially prevalent when adipocere forms in watery environments.

2.2 Chemical Process of Formation

Adipocere has been referred to as "a variant of putrefaction" (Garland and Janaway 1989, 23; Praveen et al. 2013). Quite simply, it is the substance that forms from the hydrolysis and hydrogenation of fats in the body (Mant and Furbank 1957, 32). Adipose cells are largely composed of triglycerides, which are fatty acids bound together by glycerol molecules (Praveen et al. 2013). Throughout decomposition, triglycerides are cleaved of their glycerol molecules through hydrolytic bacterial enzymes to produce free fatty acids, both saturated and unsaturated, until there are far fewer triglycerides compared to free fatty acids (Evans 1963; Forbes et al. 2004). Hydrolysis during the early stages of decomposition is initiated by intrinsic lipases, then is continued by bacterial enzymes (e.g. *C. perfringens*) (Evans 1963, 52; Ueland et al. 2014). Through hydrogenation, the unsaturated fatty acids (i.e. palmitoleic, oleic, and linoleic) are converted to the saturated fatty acids myristic, palmitic, and stearic acid (Forbes et al. 2004). These saturated fatty acids make up the majority of adipocere (Forbes et al. 2004; Ubelaker and Zarenko 2010; Ueland et al. 2014). Unsaturated fatty acids are present in smaller amounts depending on the advancement of adipocere formation (Ueland et al. 2014). Other processes that occur during hydrolysis and hydrogenation include the "attachment" of free fatty acids to sodium and potassium ions found in interstitial fluid and the cells themselves as cell membranes break down, respectively (Forbes et al. 2004, 566; Gill-King 1997). Sodium ions result in adipocere that is crumbly in texture, potassium ions produce a pasty consistency (Gill-King 1997). In a mineral-rich environment (i.e. water or soil), the sodium and potassium ions can be replaced by calcium ions to produce calcium salts of fatty acids, and a subsequent hardening of the adipocere (Gill-King 1997; Forbes et al. 2004, 566). Magnesium ions may also be present in these mineral-rich environments and can also replace sodium and potassium ions (Forbes et al. 2005c). When these calcium and magnesium ions are "conjugated" (194) with hydrolyzed fatty acids, insoluble soaps are formed in the adipocere (Takatori 2001). Hydroxy fatty acids can also form enzymatically from oleic acid, specifically through the saturation of double bonds (Takatori and Yamaoka 1977; Takatori et al. 1986; Yan et al. 2001). While bacterial activity converts fatty acids into hydroxyl fatty acids, it produces ammonia-rich waste and cell destruction, creating a more alkaline environment in which bacterial growth, and thereby putrefaction, is reduced (Ueland et al. 2014, 380). Bacterial activity also slows down as moisture in the body is lost to hydrolysis and tissues dehydrate (Evans 1963; Forbes et al. 2011). Eventually, gram-negative bacteria will take over as adipocere formation advances, inhibiting further lipolysis (Ueland et al. 2014).

2.3 Saponification Theory

The process of saponification has often been used interchangeably with that of adipocere formation (see Mant 1987 and Gill-King 1997 for examples). This is no longer the case. The saponification theory "states that the main reaction of formation [in adipocere] is the hydrolysis of fat into fatty acids and glycerol. Insoluble soaps of the fatty acids are then formed" (Magni et al. 2021, 3). The specific process involves the reaction of unsaturated fatty acids with hydrogen to produce hydroxystearic, hydroxypalmitic, and other stearic compounds (Praveen et al. 2013, 52). Since soaps, in the form of fatty acid salts, only compose a small portion of adipocere, the saponification theory is not sufficient to explain adipocere formation (Berueuter et al. 1996; Takatori 2001). Specifically, soaps only compose about 2-3% of the adipocere product (Magni et al. 2021). Fiedler and Graw (2003) indicate that saponification mostly occurs in water. This makes sense given that, in conjunction with lipids, "water reduces the rate of oxidation of fatty acids, thus making them available for binding by salts at low pH" (Gill-King 1997, 94).

2.4 Factors Contributing to Adipocere Formation

2.4.1 Moisture and Humidity

Moisture is essential for adipocere formation, since water is required for hydrolysis, but there is sufficient water in the body to enable its formation without any external contributions (Mant 1957; Janaway and Garland 1989; Forbes et al. 2005c; Widya et al. 2011; Ubelaker and

Zarenko 2010). A damp environment, as opposed to a dry or completely submerged one, appears to be the most conducive to adipocere formation (Mant and Furbank 1957, 34). While adipocere will form in dry environments, it does so at a significantly reduced rate (Magni et al. 2021). Furthermore, Forbes et al. (2005a) found that both saturated and dry burial environments produce adipocere, though it is more complete in a saturated burial environment. Fog or haze at the time of inhumation in a vault context appears to result in a higher incidence of adipocere formation, no matter the temperature of the season (Evans 1963, 52). Lastly, mass graves tend to support adipocere formation by trapping moisture between bodies, with the most adipocere found in the middle of the pile (Magni et al. 2021; Mant 1987).

2.4.2 Submergence

Though many factors are at play, a body that has copious adipose tissue and is submerged for an extended length of time will likely form adipocere (Gill-King 1997, 103). Alkaline water appears to be the most ideal for adipocere formation (Ubelaker and Zarenko 2010; Hanganu et al. 2017). Aquatic and waterlogged settings, which have a low redox potential and are therefore more anaerobic, tend to also encourage adipocere formation (Gill-King 1997). In a study conducted by Alfsdotter and Petaros (2021) in the cold climate of Sweden, all surface remains exposed for more than 22 months to the elements had completely skeletonized, but the same did not hold for aquatic remains. Alfsdotter and Petaros (2021) also determined that remains found in aquatic environments often failed to skeletonize completely due to the presence of adipocere, a trend not seen among terrestrial cases. Only in one aquatic case from this study, where the individual had been deceased for 77 years, did the remains skeletonize. This potentially indicates that adipocere forms more consistently on submerged remains. Indeed, experimental studies on pig cadavers by Yan et al. (2001) indicate that saturation of oleic acid is fastest in distilled water, speeding up adipocere formation by producing hydroxy fatty acids. While adipocere can technically form in fresh, saline, or chlorinated water, factors such as temperature, bacterial community, and scavengers make it more or less likely to occur (Widya et al. 2011).

2.4.3 Salinity

Water with high salinity tends to limit bacterial activity and thus adipocere formation (Widya et al. 2011; Alfsdotter and Petaros 2021; Magni et al. 2021). Salinity also slows down the saturation of oleic acid, and subsequently the formation of hydroxy fatty acids (Yan et al. 2001). Despite these assertions, Simonsen (1977) reports a case study of an individual who washed up on a beach by the North Sea, covered in adipocere, after only 22 days postmortem. Furthermore, Mant and Furbank (1957) cite Sir Sidney Smith who, in 1955, concluded through experimental work that adipocere formed in salt water as well as distilled. Finally, Takatori and Yamaoka (1977) report adipocere on 3 bodies pulled from the sea after 3-6 months estimated postmortem. Therefore, there are several conflicting accounts within the literature.

2.4.4 Chlorine

High chlorine content can significantly reduce bacterial activity, and thus adipocere formation (Alfsdotter and Petaros 2021). However, Yan et al. (2001) found that there is an increase in degradation of oleic acid after 6 weeks, indicating that it may just take longer for adipocere to form in a chlorinated environment. Despite chlorine's ability to limit bacterial activity, Stuart et al. (2016) report that adipocere formation was enhanced in chlorinated water. The authors note, however, that these results may arise from the formation of chlorohydrin as hypochlorous acid reacts with unsaturated bonds in adipose tissue (Stuart et al. 2016). Chlorohydrin would increase as oleic acid decreases and stearic acid increases, mimicking an advanced stage of adipocere formation (Stuart et al. 2016; Magni et al. 2021).

2.4.5 Aquatic Depth

Depth of submersion does not seem to be a significant factor in adipocere formation (Forbes et al. 2011). Nevertheless, complete submersion of remains can limit insect and scavenger activity, thereby creating a more ideal condition for adipocere formation (Magni et al. 2021). When a body is partially submerged, adipocere will tend to form 2 inches above and 2 inches below the water line about 2-4 weeks postmortem (Bass 1997, 184).

2.4.6 Temperature

When discussing the relationship between temperature and adipocere, it is often useful to consider both terrestrial and aquatic contexts. The ideal range in which bacterial activity, and subsequently adipocere formation, is greatest is at 22-40 ℃ on land (Magni et al. 2021) and 21-45 ℃ in water (Widya et al. 2011). Outside these ranges, adipocere may still form, even at temperatures as low as 4-20 ℃ or as high as 40 ℃ (Forbes et al. 2005a), but at a significantly slower rate, to the point where it may cease altogether even if formation has already begun (Magni et al. 2021; Forbes et al. 2011; Ubelaker and Zarenko 2010; Alfsdotter and Petaros 2021). Specifically, Magni et al. (2021) indicate that if temperatures dip below 7 ℃ that adipocere formation will stop. In experimental studies, Forbes et al. (2005a) found that a warm (40 ℃) burial environment produced adipocere, but even after 12 months, adipocere was not observed in a cold (4 ℃) burial environment. In an aquatic context, if the water is too warm soft tissue will break down too quickly; if it is too cold, all moisture within the body will freeze (O’Brien 1997) limiting the amount available for hydrolysis.

2.4.7 Oxygen

In general, anaerobic conditions are more conducive to adipocere formation (Yan et al. 2001; Forbes et al. 2005a; Praveen et al. 2013). Experimental and field studies tend to support this claim. Mant and Furbank (1957) found that disturbing bodies that had been buried before adipocere or mummification could develop "prolonged the period of colliquative putrefaction" (22), preventing adipocere formation. They also found that within ditch water, anaerobic conditions were "slightly more conducive to adipocere formation than aerobic conditions" (Mant and Furbank 1957, 26). Forbes et al. (2005a) found that after a 12-month period, an aerobic burial environment did not result in adipocere formation, whereas an anaerobic burial environment did produce adipocere. They suggest that this is because an aerobic environment favors rapid decomposition. However, Takatori et al. (1986) determined from laboratory studies involving bacteria that an anaerobic environment was not strictly necessary for adipocere formation given the ability of some aerobic bacteria to hydrolyze fatty acids in the presence of dissolved oxygen.

2.4.8 Body Composition

A body presenting with more adipose tissue is more likely to develop adipocere (Mant and Furbank 1957; Ubelaker and Zarenko 2010; Widya et al. 2011), especially around the abdomen, buttocks, cheeks, breasts, and feet, though only when still covered by shoes (Magni et al. 2021). Subsequently, adipocere occurs more often in children, biological females, and obese individuals (Gill-King 1997; Ubelaker and Zarenko 2010; Teo et al. 2014; Hanganu et al. 2017). This is highlighted by a case study described by Pfeiffer et al. (1998) wherein two individuals, a male and female, were buried together but only the female displayed adipocere. This is because, through lipolysis, the more adipose tissue present at death, the more free fatty acids can be converted to adipocere. However, adipocere has also been known to form on defleshed bones (Moses 2012), and isolated body parts and organs (Mant 1987; Forbes et al. 2011; Hanganu et al. 2017).

2.4.9 Soil

Soil type appears to play a significant role in the chemical composition of adipocere (Forbes et al. 2002). Generally, more alkaline (pH of 5-9) soils are conducive to adipocere formation (Forbes et al. 2005a; Ubelaker and Zarenko 2010; Jopp-van Well et al. 2016; Magni et al. 2021). However, Forbes et al. (2005a) demonstrated that adipocere can still form in more acidic burial environments, but the progress of formation is substantially reduced. Soils such as clay and loam that retain moisture are considered ideal for adipocere formation (Rodriguez 1997; Ubelaker and Zarenko 2010; Magni et al. 2021). In experimental studies, Forbes et al. (2005c) found that clay, sterilized, and control soils, where the sterilized and control soils consisted of a loamy sand soil made up of quartz, some anorthite, and a little illite - a secondary clay mineral – formed adipocere, but the adipocere was less stable under these conditions. The clay soil consisted largely of quartz, but also consisted of secondary clay minerals such as illite, kaolinite, and montmorillonite (Forbes et al. 2005c). There is some debate over whether sandy and silty soils are also ideal mediums since they are considered well-draining soils, and it seems that other factors, like whether the sand and silt are situated next to a body of water, play a role here (Magni et al. 2021; Ubelaker and Zarenko 2010). Forbes et al. (2005c) have even shown that advanced adipocere formation can occur in sand and silty sand conditions, though it is important to note that during their experiments they kept the soils temperate and moist. The sand and silty sand in this instance were found to be made up mostly of quartz, with potentially small amounts of iron oxides or kaolinite (Forbes et al. 2005c). Some authors have claimed that well-drained soils are actually more conducive to mummification (Mant 1987; Mant and Furbank 1957), but mummification is not mutually exclusive of adipocere. Hanganu et al. (2017) indicate that the presence of groundwater may also help determine if adipocere forms on a body.

2.4.10 Depth of Burial

In assessing how depth of burial impacts adipocere formation, some general observations regarding decomposition are insightful. Decomposition is often more advanced the longer the period between death and burial (Mant and Furbank 1957). Furthermore, a body decomposes faster on the surface compared to buried remains, largely due to insect and scavenger access (Janaway and Garland 1989). Indeed, in a case review by Alfsdotter and Petaros (2021), all three instances of buried remains were covered in adipocere and had been buried for 2-5 years. Mant (1987, 69) explains that "below a certain depth the temperature will remain constant at a low temperature, thus acting as a cooling chamber or refrigerator." Deep burials also tend to be wet, due to less evaporation, so there is a tendency to find more adipocere formation (Rodriguez 1997). However, Vane and Trick (2005, 22) found that burial depth did not necessarily correlate to stage of adipocere formation or "extent of fat decay" in a case study of a bovine and porcine mass burial. The authors attributed this to the variation in fatty acid profiles between cattle, pigs, and humans, as well as the presence of clay infill between layers of carcasses and a subsequently perched water table. Overall, depth may only matter in the case of a shallow burial.

2.4.11 Body Coverings

It is clear from the literature that clothing or coverings on a body enhances adipocere formation (Mant and Furbank 1957; Mant 1987; Forbes et al. 2005b). Coffins, however, tend to limit adipocere formation due to their aerobic environments (Forbes et al. 2005b). Coffins also limit cation exchange with soils, preventing calcium and magnesium salts of fatty acids from forming (Forbes et al. 2005b). Overall, this significantly slows down adipocere formation. This is supported by field work conducted by Mant (1987) wherein bodies buried with coffins that had more airspace decomposed faster than those without a coffin. In particular, clothing and coverings that trap and hold moisture against the skin are more conducive to adipocere formation in all environments (Ubelaker and Zarekno 2010; Hanganu et al. 2017; Magni et al. 2021). Magni et al. (2021) argue that natural fibers like cotton and wool that absorb moisture are better for increasing adipocere formation, especially in aquatic environments, so long as the fabric is not so thin that it disintegrates (Magni et al. 2021). Indeed, Forbes et al. (2005b) found that after a 12-month period of burial in a coffin, cotton clothing disintegrated and the human remains extensively decomposed.

However, some studies seem to suggest that synthetic fibers are also conducive to adipocere formation. Fiedler et al. (2004) conducted a study of two exhumed corpses from a cemetery that was established in 1974 and found that the bodies presented with extensive adipocere formation, but were clothed in both natural and synthetic materials. Manhein (1997) presents another case in which a woman was found wrapped in a polyester mattress cover, wearing clothes, and buried about 3 feet deep for 8 years with adipocere formation. However, water appeared to occasionally seep into the grave, so this may also play a role. Finally, Forbes et al. (2005c) found that adipocere formed on remains clothed in polyester that were buried in a coffin for 12 months. It is unclear if plastic wrappings encourage or discourage adipocere formation since Jopp-van Well et al. (2016) indicate that both plastic and concrete increase adipocere formation, and Alfsdotter and Petaros (2021) concur, given that anoxic conditions are beneficial for anaerobic bacteria. In contrast, Magni et al. (2021) report that results differ, and Forbes et al. (2005b) observed that plastic wrapping resulted in rapid decomposition and liquefaction of remains. Forbes et al.(2005b) note, however, that in an unofficial study of remains wrapped in clothing, then wrapped in plastic, that adipocere was able to form. Manhein (1997, 471) reports a case wherein a man was wrapped in plastic and buried 3 feet deep for 5 years and presented with "extensive adipocere," but it was not stated if the individual was clothed.

2.4.12 Seasonality

There is little discussion of seasonality in the literature. Evans (1963, 52) found that individuals interred in vaults in the "second and third quarters of the year," when temperatures were higher, displayed more adipocere compared to those who were interred during colder seasons. Mant (1987) also comments that bodies buried in warmer months decompose faster than those buried in colder seasons, but he does not indicate how this may influence adipocere formation.

2.4.13 Bacteria

Particularly in the earliest stages of adipocere formation, bacteria are "essential" (Mant and Furbank 1957, 34). In experimental studies, tissues exposed to a solution with *Clostridium welchii* (*perfringens*) showed fat hydrolysis, whereas tissues exposed to a sterile solution could not undergo hydrolysis (Mant and Furbank 1957, 27). Interestingly, Forbes et al. (2005c) found that adipocere still formed in sterilized soil conditions, but they indicate that the soil was sterilized to "destroy most of the soil bacteria" (36). As Ueland et al. (2014) and Pfeiffer et al. (1998) discuss, gram-positive bacteria (e.g. *Bacillus*, *Nocarida*, and *Cellulomonas*) are involved in lipolysis, and gram-negative bacteria (e.g. *Pseudomonas*, *Serratia*, *Alcaligenes,* and *Enterobacter*) aid in the continued formation of adipocere, with *Pseudomonas* and *Enterobacter* also found in aquatic samples. Two gram-positive, anaerobic bacteria *Clostridium perfringens* (formerly *Cl. welchii*) - which originates in the bowel (Mant and Furbank 1957; Kumar et al. 2009) - and *Cl. frigidicanes* are especially important since they produce the enzyme lecithinase that assists in hydrolyzing and hydrogenating fat tissue (Kumar et al. 2009; Teo et al. 2014;

Hanganu et al. 2017). *Clostridium perfringens* replicates slowly at temperatures below 70°F, so "very rapid initial cooling" (34) of the body may prevent adipocere formation (Mant and Furbank, 1957). *Clostridium perfringens* is also a key player in the production of hydroxy fatty acids, which are important for adipocere formation and stabilization in the internal organs (Takatori et al. 1986). Another bacterium of note is *Micrococcus luteus*, an aerobic bacterium that can hydrolyze fat into fatty acids and "convert oleic acid to hydroxy and oxo fatty acids" (Takatori et al. 1987, 10). This demonstrates the importance of both anaerobic and aerobic bacteria in the formation of adipocere.

2.4.14 Micro-Climates

Because the conditions under which adipocere forms can be so variable and unpredictable, a situation may arise wherein a micro-climate is created somewhere on the dead body or in a specific environment that facilitates adipocere formation. One example is the climate created by certain materials against the skin, such as leather, that enable the body to retain moisture in an otherwise dry environment (Magni et al. 2021). There are also instances of adipocere on bodies that have been found inside cars submerged under water (Magni et al. 2021).

2.4.15 Desiccation

While it was once thought that a body could not undergo multiple types of decomposition, it is now clear that "...the changes of saponification, mummification and putrefaction may all be seen on the same cadaver" (Mant 1987, 65; Praveen et al. 2013). In fact, Bereuter et al. (1996, 268) calls adipocere a "fatty wax type of mummification," and Ubelaker and Zarenko (2010, 170) refer to it as a "chemical type of mummification." Evans (1963, 51) even reports that bodies interred in a dry vault for an extended period of time (e.g. 100 or more years) and demonstrating adipocere formation also tended to have desiccated non-adipose tissue. However, Janaway and Garland (1989) make the clear distinction that mummification should only refer to artificial preservation, and it is instead more accurate to call natural forms of the process "desiccation."

2.5 Timing

The timing of adipocere formation can vary significantly based on the environmental conditions described above. The factors that are particularly important appear to be temperature and whether a body is on land or in the water. Interestingly, Mant and Furbank (1957, 18) point out that, though adipocere has long been considered a "phenomenon of late decomposition," it can actually begin to form soon after death. This statement is supported by Moses (2012) who indicates that adipocere is macroscopically visible at six weeks postmortem but begins forming within hours of death. According to Ubelaker and Zarenko (2011), adipocere is macroscopically visible after 1-3 months, but this can vary. Other accounts state that adipocere forms in 3-6 months, with partial conversion at six weeks, but that it can take years to inundate the entire body (Bereuter et al. 1996), though one paper clarifies that it can take up to two years to reach "complete formation" (Magni et al. 2021, 8). Hanganu et al. (2017) generally concur but specify that adipocere formation begins in the skin at six weeks and extends to the muscles after 3-4 months. In a survey of North American forensic anthropologists, it was reported that adipocere was visible as early as 2-3 weeks postmortem (Manhein 1997). Some researchers even cite that adipocere can appear after only a few days in a "warm, moist environment," but that it takes longer in a hot, dry environment (Gill-King 1997, 101; Magni et al. 2021). While Kumar et al. (2009) argue that adipocere can form anywhere from three days to five years they report on a case wherein a body found in a "marshy pond" (475) presented with macroscopic adipocere formation after only 65-66 hours postmortem. They attribute this rapid formation to the marshy environment, an average temperature of 30-35 ℃ with 80-90% humidity during the month the body was recovered, as well as sufficient adiposity of the body and the presence of clothing.

Fully submerged remains tend to develop adipocere after an extended period of time compared to terrestrial environments, though it can range from 3 weeks to 5 years (Rodriguez 1997, 461). Experimental work conducted with tap water showed that at a mild temperature of 60-70 °F (~16-21 ℃) adipocere was observable at 2-3 months, and "well formed" at 18 months (Mellen 1993, 92). The same experiment also demonstrated that at 40 °F (~4 ℃) it took 12-18 months for adipocere to form. In freshwater environments, adipocere formation also appears to occur after 2-3 months (Widya et al. 2011; Forbes et al. 2011; Alfsdotter and Petaros 2021) but, again, that it takes 12-18 months if the water is cold (Forbes et al. 2011). According to Alfsdotter and Petaros (2021), this slight discrepancy between terrestrial and aquatic contexts is because decomposition is slower in water. Interestingly, in a review of Swedish autopsy cases, adipocere formed more often on submerged remains, especially if the remains were submerged for more than one year (Alfsdotter and Petaros 2021). Thus, adipocere appears to form more slowly, but more consistently, on submerged remains. This appears to not always be the case, however, since Simonsen (1977) reports a case of a man found washed up on a beach on the North Sea who presented with adipocere after only 22 days postmortem, though the weather conditions during the individual's absence were considered ideal in this case. Unfortunately, though Forbes et al. (2004) identified stages of adipocere formation based primarily upon the ratio of saturated to unsaturated fatty acids in adipocere samples, these stages could not be correlated to a fixed time frame and were highly dependent upon environmental conditions. Work by Moses (2012) supports this conclusion. Therefore, while adipocere can be broken down into stages of formation, this is not a reliable indicator of the postmortem interval.

2.6 Preservation

From a forensic and bioarchaeological perspective, soft tissue preservation often seen in conjunction with adipocere is of paramount interest. Overall, decomposition is slowed in the areas where adipocere forms on the body and as it hardens over time (Ueland et al. 2014; Magni et al. 2021). This is because adipocere is a stable form of fat (Ueland et al. 2014), though it may also help that tissues adjacent to adipocere often desiccate (Mant 1987) and that it can limit insect and scavenger activity (Teo et al. 2014). Indeed, adipocere can persist for hundreds or even thousands of years (Mant and Furbank 1957), only breaking down quickly if exposed to air, moisture, fungus, or certain bacteria (Ubelaker and Zarenko 2010). In case studies, Mant and Furbank (1957) observed five bodies exhumed from a London church that had extensive adipocere formation and well-preserved organs, even after a 100 year-long internment. Pfeiffer et al. (1998) report a case of adipocere lasting for 120 years. Finally, Fiedler et al. (2009) found an adipocere-like crust on a child who had been deceased for 1600 years; the authors attributed the persistence of the adipocere to fluctuating groundwater levels that maintained an anaerobic burial environment.

2.6.1 What Makes Adipocere Stable

Adipocere is considered chemically stable once all the unsaturated, oleic fatty acid has been completely hydrogenated and is no longer present in the sample (Forbes et al. 2005c). Furthermore, hydrogenation and dehydrogenation that result in 10-hydroxy and 10-oxo fatty acids, respectively, raise the melting of fat, also stabilizing adipocere (Moses 2012, 590). In other words, hydroxy fatty acids possess higher melting points (Takatori and Yamaoka 1977).

Also, late-stage adipocere contains a higher concentration of calcium and magnesium salts of fatty acids, which Forbes et al. (2005c) indicate results in a hard, brittle texture.

Papageorgopoulou et al. (2010) discuss the case of a preserved medieval infant brain found in watery, acidic, clay soil. The authors indicate that adipocere is likely the cause of the excellent preservation. Correspondingly, Forbes et al. (2005c,a) found that there was an association between higher levels of oleic and 10-hydroxy stearic acids and cold, acidic, clay environments. There is also a connection between early stage adipocere formation and higher levels of oleic acid. This implies that adipocere may not need to be completely formed to still possess preservational properties, and that it is possible for adipocere to form and have a significant effect upon remains in a less than ideal environment.

2.7 Post-Mortem Interval

The post-mortem interval is simply the time that has elapsed since an individual died. A broad estimation includes the date a decedent was last seen alive, to the time when their body is recovered. Estimating this time frame is notoriously difficult in forensic contexts, since it is influenced by a variety of factors, including temperature, insect activity, humidity, soil composition, and scavenger presence (Franceschetti et al. 2023; Steadman 2009). While there are various methods for determining PMI, such as internal temperature and entomological succession, the longer an individual has been deceased, the more challenging it is to determine the PMI (Sutherland et al. 2013; Teo et al. 2014). Calculating the accumulated degree days (ADD) for a body can help determine PMI by controlling for the variable of temperature, since temperature significantly influences decomposition (Sutherland et al. 2013). In essence, ADD consists of "the average daily temperatures between the date of death (i.e. last seen alive) and the date of discovery" (Alfsdotter and Petaros 2021, 1351). Specifically, ADD is the "heat energy units" (258) that make up how much thermal energy is required for "the chemical and biological reactions that take place in the body during decomposition" (Sutherland et al. 2013, 258). However, this method may not be reliable when abnormal decomposition occurs (e.g. adipocere) or if a body has been exposed to low temperatures before burial (Pittner et al. 2020).

An accurate estimation of the PMI is often crucial to building criminal cases by shedding light on the cause and manner of death through the analysis of peri- and post-mortem trauma, determining where an individual died, and by verifying witness statements and alibis (Sutherland et al. 2013; Teo et al. 2014; Magni et al. 2021). It is also helpful during the pursuit of an unknown decedent's identity as it can limit the pool of potential candidates (Sutherland et al. 2013). Post-mortem interval estimations may fall on the forensic anthropologist if the remains are in an advanced stage of decomposition. In a forensic context, adipocere represents just one element, but it can have a significant bearing on a case. Given that adipocere can preserve remains for hundreds or thousands of years, it may also obscure dating estimations within a bioarchaeological context. It is important for these researchers to understand the effect of adipocere should they encounter it in the field.

In general, adipocere tends to obscure time of death estimates (Praveen et al. 2013), primarily by preserving remains and slowing down soft tissue decomposition (Ubelaker and Zarenko 2010; Widya et al. 2011; Magni et al. 2021). Despite the confounding influence of adipocere on decomposition, there is a dearth of methods for quantifying and qualifying its presence on a body. Alfsdotter and Petaros (2021) describe two prominent decompositional scoring methods that nonetheless fail to incorporate adipocere formation into their estimates. The Megyesi et al. (2005) method, developed in the USA, only lists saponification as a form of mummification, and only in terrestrial settings (Alfsdotter and Petaros 2021). The Heaton et al.

(2010) method from the UK includes it, to an extent: with this method, the head can be comprehensively scored, and the trunk can be scored for initial formation, but the limbs cannot be scored at all (Alfsdotter and Petaros 2021). A study conducted by Alfsdotter and Petaros (2021) demonstrates that adipocere hinders PMI estimates by making it more difficult to score decomposition.

Some methods have been mentioned in the literature for ascertaining PMI in the presence of adipocere. Mant and Furbank (1957, 18) cite Fourcroy (1789) who indicates that after 3-5 years interred, an adipocerous body will still possess “pigmented muscle groups,” but that 30-40 years postmortem these soft tissues will no longer be present. However, this is disputed by a case study of an embalmed Civil War-era soldier detailed by Bass in Rathbun and Buikstra (1984, 137), wherein pink-hued flesh was still visible after over 100 years. This highlights the variability and complexity involved in establishing a timeframe regarding adipocere formation. Another macroscopic method is the presence of “cheese skipper” (*Piophila casei*) flies that are drawn to the cheesy odor of adipocere (Magni et al. 2021, 4). These flies appear around 90-336 days after death, establishing a PMI of 3-11 months when present on a corpse (Magni et al. 2021).

There are also chemical methods for estimating PMI. The ratio of epicoprostanol to cholesterol in adipocere may be useful, with more epicoprostonal indicating a longer PMI (Ubelaker and Zarenko 2017; Hanganu et al. 2017). The presence of oxo fatty acids may indicate older adipocere (i.e. more than three months), but since they only compose about 1-2% of adipocere it may not be an entirely reliable method (Magni et al. 2021). Forbes et al. (2004) and Magni et al. (2021) found that the total saturated fatty acid composition of adipocere can indicate stage of formation, but that this does not necessarily correlate to a specific timeframe. For submerged remains, Magni et al. (2021) point to increased amounts of hydroxystearic acid compared to oleic acid as an indicator of a longer PMI, but they emphasize that this needs to be confirmed with human samples.

2.8 Chemical Makeup

A review of the specific components of adipocere will be helpful for understanding how concentrations can vary based on environment and timeframe. Gas chromatography mass spectrometry (GC-MS) is useful for identifying adipocere in low concentrations (Forbes et al. 2002). In general, Forbes et al. (2004, 2005c) found during experimental studies that the percentage of oleic fatty acid goes down as the percentage of palmitic fatty acid increases, helping establish phases of adipocere formation, and that soil type alters its chemical composition.

2.8.1 Saturated Fatty Acids

Adipocere mostly consists of the saturated fatty acids myristic, palmitic, and stearic acids (Forbes et al. 2005a,c). Palmitic acid appears to be the most abundant in adipocere, followed by stearic acid (Forbes et al. 2005a,b,c). In terrestrial studies carried out on buried pig remains, total saturated fatty acid concentration helped clarify the stage of adipocere formation, with 40-60% indicating initial, 70-90% intermediate, and 90% or more with an advanced stage of formation (Magni et al. 2021).

2.8.2 Unsaturated Fatty Acids

The unsaturated fatty acids found in adipocere include oleic, palmitoleic, and linoleic acids, which are liquid at room temperature (Takatori 2001; Kumar et al. 2009). If any unsaturated fatty acids remain in an adipocere sample after complete conversion, it is typically oleic acid (Forbes et al. 2005b). Early stage adipocere formation is characterized by >20% oleic acid, and later stages <10% oleic acid (Yan et al. 2001; Vane and Trick 2005). Though these findings come from a study of submerged remains, Vane and Trick (2005) validate the resuults with an analysis of adipocere from a 1967 mass grave of cattle and pig carcasses, wherein they found the adipocere from 50-70 cm deep consisted of 5.1% oleic acid.

2.8.3 Hydroxy Fatty Acids

The hydroxy fatty acids found in adipocere are 10-hydroxy fatty acids only, including 10-hydroxy stearic acid and 10-hydroxy-12-octadecenoic acid (Takatori and Yamaoka 1977; Takatori et al. 1986; Takatori 2001). These form as a result of bacterial activity (Takatori et al. 1986). For submerged remains, Magni et al. (2021) point to increased amounts of hydroxystearic acid compared to oleic acid as an indicator of a longer PMI, but they state that these results need to be confirmed with human samples. Forbes et al. (2002) found in cemetery and forensic burial contexts that only the wet samples contained 10-hydroxy stearic acid, with high palmitic acid concentrations and lower stearic acid concentrations. Experimental studies by Takatori et al. (1986) however, found that hydroxy fatty acids could form under a variety of environments, including water or soil.

2.8.4 Oxo Fatty Acids

Similar to hydroxy fatty acids, only 10-oxo fatty acids have been found in adipocere (Takatori and Yamaoka 1977), and they are not found in all instances of adipocere (Takatori 2001). They also only make up a small portion of adipocere (Takatori 2001), about 1-2% (Magni et al. 2021). Oxo fatty acids are the result of bacterial activity (Takatori et al. 1986). The presence of oxo fatty acids may indicate that adipocere is more than 3 months old (Magni et al. 2021).

2.8.5 Salts of Fatty Acids

The salts of fatty acids found in adipocere include sodium, potassium, calcium, and magnesium (Gill-King 1997). Initially upon death, sodium salts of fatty acids form, followed by potassium salts of fatty acids which increase in concentration from the potassium found in cell membranes (Forbes et al. 2005c). As decomposition progresses, sodium and potassium ions in the body are replaced by minerals present in the surrounding environment, this leads to the formation and presence of calcium and magnesium salts of fatty acids in increasingly higher concentrations (Forbes et al. 2005c, 41). These findings were determined based on a 12-month experimental study of adipocere formation in various types of soils.

Clearly, there exists substantive research on the formation, composition, and properties of adipocere. Despite this, there exists no method for systematically estimating PMI based on this information. For the most part, this is because of a plethora of confounding variables that influence rate, degree, and quality of adipocere formation. This includes the categories already discussed, such as environmental temperature, soil type, pH, clothing, and much more. Combining these variables to create a predictive model for PMI estimation based on adipocere - instead of in spite of it - could represent a valuable tool for forensic investigators. However, subjective, non-standardized terminology (e.g. “lard-like” in Hobischak 1994) makes it difficult to begin the process of collating diverse research in a comprehensive manner. As does a common theme of underreporting key variables, such as the temperature of a context or the texture of adipocere. This thesis is a major step in changing how researchers study and report on adipocere.

3 MATERIALS AND METHODS

3.1 Materials

The program used to build the searchable database (Appendix B) presented in this thesis (henceforth called the "database") and a preliminary spreadsheet of data (Appendix A) is Microsoft Excel for Microsoft 365 MSO (Version 2308). Data come from original research articles dating from 1977 to 2016 that report experimental test results on human and porcine remains, and case studies of human remains recovered from aquatic and terrestrial environments. Of note, the literature review incorporates a wider range of sources than were ultimately included in the database. A preliminary spreadsheet was compiled to systematically catalog the recorded variables for each study. This spreadsheet was referenced in determining the final samples included in the search engine database, along with the articles themselves to ensure accuracy in reporting the details of each case. Sources were not included in the preliminary spreadsheet if they did not report a sufficient number of variables compared to the majority of the sources and a known or assumed PMI. For example, Alfsdotter and Petaros (2021) report on decomposition trends based on a large study of cases in Sweden. However, details such as temperature, time of year, soil type, and presence of clothing were not provided for individual samples.

The preliminary spreadsheet contains information from 9 case study articles ($n = 57$ individual samples), 13 experimental studies ($n = 107$ individual samples), and 2 long-term studies ($n = 2$ individual samples). The final search engine database contains N = 16 separate sources, $n = 19$ freshwater cases, $n = 41$ terrestrial shallow burial cases, and $n = 16$ marine cases. These contexts – freshwater, marine, and terrestrial shallow burial – were chosen because they are best represented in the preliminary spreadsheet and because they are common forensic contexts.

Samples classified as “long-term” such as those in Fiedler et al. (2009) and Papageorgopoulou et al. (2010) that are 1600 to 800 years old, respectively, are excluded from consideration at the current time because the sample size is too small, and the minimum PMI is not useful in a medicolegal context. In the future, this database may also be of use to bioarchaeologists who encounter adipocere in a historic or prehistoric context. Work by Moses (2012) and Jopp-van Well et al. (2016) demonstrate that adipocere forms within bone, but there is currently no reliable methodology for ascertaining PMI for these remains.

3.1.1 Porcine Sampling

Porcine remains are often used as a proxy for human cadavers in experimental studies. Despite this, in experimental work comparing human and pig decomposition, Connor et al. (2018, 1354) found that “the trajectory of decomposition observed between human and pig samples diverged in rate and gross presentation,” and they proceed to warn readers that “caution is warranted when attempting to apply data derived from pigs to human subjects” (1354). Dautartas et al. (2018, 1682) echo this sentiment, and found in a study comparing human, pig, and rabbit decomposition that “human remains behave less predictably than those of pigs or rabbits such that nonhuman models could not replicate the impacts of differential insect activity, scavenging or physical state changes (e.g. mummification) exhibited by the human subjects.” They also say that using nonhuman subjects will result in more frequent errors. Matuszewski et al. (2020) disagree, indicating that pigs are more reliable in entomological and taphonomy studies, especially given that experiments using pig cadavers are easily replicated, more easily meet cadaver requirements, and certain factors are more easily controlled (e.g. body mass) compared to human cadavers that are in comparatively short supply. The authors also indicate that pigs are similar to humans in some key ways, including body mass, general anatomy, body composition, gut microbiota, and gross processes of decay (803). Sutherland et al. (2013) concur that pigs have similar digestive tracts and intestinal flora. Furthermore, studies using human samples are usually small, limiting the statistical power of any results (Matuszewski et al. 2020). Matuszewski et al. (2020, 808) acknowledge the studies that indicate that pigs are not adequate proxies for human cadavers in studies of decomposition, but that "there is no better candidate at this time." This conclusion by Matuszewski et al. (2020), and the overwhelming number of studies conducted using pigs, make it impractical to exclude these studies from this paper. Data drawn from experiments using pig corpses or tissues, however, are notated as such.

3.2 Methods

This paper uses data obtained from original research articles to develop a model of a search-engine based catalog, presented here in the form of a searchable Excel database. Given the practical restrictions of conducting an experiment on adipocere that is both macro- and microscopic in its focus, the decision was made to conduct a literature-based study. Only cases with known PMI (or last known date an individual was seen alive) were eligible for entry into the database. No set number of variables were required for entry into the database, though basic context (e.g. terrestrial, burial, freshwater, marine) was one essential variable. According to Pittner et al. (2020), even forensic entomology only provides a minimum PMI, and is only reliable up to six weeks postmortem. The database presented in this paper possesses several samples that are in an advanced stage of decomposition, and therefore for which other methods would not be reliable.

To create the searchable database, each source was assigned a unique identifier (Table 1). The source was then referenced, along with a preliminary spreadsheet, to input the information for each variable. For some entries (E2, E3, E9, CS1, CS3) certain data were extrapolated from the original source. Most often this is in the form of temperature when an article indicates the year, month, and specific region that an experiment or case study took place. A third-party website was then referenced to estimate a temperature range. In one case (CS1), two deceased individuals were found wearing wetsuits. Research indicates that wetsuits are composed of neoprene, a synthetic fiber (Education.nationalgeographic.org).

Table 1 Source Identifiers

Source	Experimental ID	Author	Case Study ID
Stuart et al. (2016)	E1	Byard (2016)	CS1
Hobischack (1994)	E2	Kumar et al. (2009)	CS2
Forbes et al. (2011)	E3	Dix (1987)	CS3
Forbes et al. (2005a)	E4	Adachi et al. (1997)	CS4
Forbes et al. (2004)	E5	Manhein (1997)	CS5
Forbes et al. (2005b)	E6	Kahana et al. (1999)	CS6
Forbes et al. (2005c)	E7	Simonsen (1977)	CS7
Rodriguez and Bass (1985)	E8		
Anderson and Hobischack (2004)	E9		

3.2.1 Variables

The variables included in the database were chosen based on three considerations: their impact on adipocere formation as determined from the literature review, reportability in both experimental and case studies, and feasibility for medicolegal and scientific experts to collect. Each variable is also assigned set input options to establish standardization in reporting (Table 2). Certain variables are elaborated upon below. For a definition of terms used, see Table 3.

Table 2 Database Variables

Variable	**Selections**
Ecosystem	Freshwater Salt Water Marine Terrestrial
Context	Lake Pond Marsh Swamp

	River Stream Drainage Ditch Irrigation Channel Sinkhole Ocean Beach Woodland Forested Open Burial Damp Burial Saturated Burial Dry Burial
Temperature*[1]	Cold (0-4℃) Cool (5-20℃) Warm (21-45℃) Spring Summer Fall Winter
U.S. State	State Name International
Soil	Loamy Sand Sand Clay Silt Silty Sand
Clothing	Naked Partially Clothed Fully Clothed Present
Fibers	Natural Synthetic Mixed
Additional Covering (AddtlCovering)	Plastic Coffin (General) Lined Coffin Unlined Coffin Blanket Wooden/Cardboard Box Car Closed Environment Styrofoam Box/Chest Sleeping Bag
Depth	Immersed/Covered Shallow (<1.2 m terrestrially) Deep (≥1.2 m terrestrially) Surface
pH	Acidic (<7.0)

	Alkaline (≥7.0) Lime
Subject	Human Adult Human Child Human Pig (Whole) Pig (Adipose Tissue)
Sex	Male Female
Adipocere Coverage (ACoverage)	Extensive Partial Minimal Abdomen Chest Upper Limbs (excluding hands) Lower Limbs (excluding feet) Face/head Cutaneous Subcutaneous
Adipocere Texture (ATexture)*[2]	Soft Slimy Hard Crumbly Greasy Waxy Fatty
Adipocere Color (AColor)	White Yellow Gray Brown Pink Green
Adipocere Odor (AOdor)	Ammoniacal Cheesy None
Fatty Acid Concentration	Total % Saturated Fatty Acids • Early (40-60%) • Intermediate (70-90%) • Advanced (>90%) Oleic Acid (Yan et al. 2001) • Early (>20%) • Late (≥10%) Linoleic Acid • Early (≥1%) • Intermediate/Advanced (<1%) Palmitoleic Acid • Early (≥1%) • Intermediate/Advanced (<1%)

	Palmitic Acid • Early/Intermediate (<50%) • Advanced (>50%)

*[1]Temperature ranges established based on the literature (Magni et al. 2021; Widya et al. 2011; Forbes et al. 2005a).

*[2]If a paper used the term “lard-like” (e.g. Hobischak 1994) it is classified as “fatty”.

Table 3 Terminology Definitions

Term	Definition
Variable	The broad factors that contribute to adipocere formation, consisting here of 22 variables (e.g. Ecosystem, Context, Temperature, etc.)
Test Study	The study reported within an academic article, the details of which are “plugged” into this paper’s adipocere database and used to validate the utility of the database in producing a PMI estimate for a case of unknown PMI.
Experiment (A-F)	When an academic article is referenced against the paper’s adipocere database to test whether the dabatase can produce a PMI estimate similar to the known PMI of the article.
Run 1	The first set of filters for a given experiment.
Run 2	The second set of filters for a given experiment.
Run 3	The third set of filters for a given experiment.
Blank	The term automatically generated in the computer program Excel when no value is input for a table cell. Indicates studies where a certain variable (e.g. temperature) was not reported, and thus no value could be input into the database.
Refined	Specific variable selections (e.g. “freshwater” for the ecosystem variable) were chosen for a run to the exclusion of other variable selections.
Unrefined	A certain variable (e.g. ecosystem) is not filtered except by other variable selections.
Unreported	A study does not provide the details for a specific variable (e.g. the temperature of a body of water), thus potentially limiting the abilitiy of a run to filter by that variable.

Regarding the category defined here as “ecosystem”, a drawback is that it is difficult to account for brackish water. Zero experimental studies were found involving brackish water, and no case studies explicitly mention it. Kumar et al. (2009) present a case with a body found in a marshy pond, but it was ultimately ruled that this would be grouped under freshwater. Furthermore, the category of saltwater is present to account for both experimental testing that is considered saline, rather than sampled from a marine body of water, and case studies identified as being recovered from saltwater contexts such as lagoons, brackish bays, and saltwater rivers. For samples taken or bodies recovered from marine (i.e. open ocean and associated shoreline) contexts, the term "marine" will be applied. This delineation is meant to better capture the differences in bacterial populations that may exist between marine bodies of water and other saltwater contexts (i.e. brackish and experimental contexts).

When it comes to "depth", unlike terrestrial burials, depth in aquatic environments is difficult to define because it depends on the body of water and the action of tides and currents. Temperature is more predictable in the ocean, depending on latitude and depth (Sciencelearn.org.nz; Oceanexplorer.noaa.gov). Freshwater bodies of water are more variable, especially as they increase in size (Bussières and Granger 2007). Such variables may include lake morphology and latitude (Bussières and Granger 2007). Efforts have been made to develop systematic methods for estimating the temperature of a given river based on regional data (Ouarda et al. 2022), but each body of water will vary depending on region. Some other factors that are taken into consideration when modeling freshwater temperature include solar radiation, air temperature, wind speed, and humidity (Risley et al. 2003). Therefore, it is difficult to establish standard depth values without considering a host of other variables. The categorization of "shallow" and "deep" are thus left up to the researcher or forensic specialist, and it is important that a temperature measurement be collected at whatever depth remains are placed or recovered. In many experimental studies, samples are merely submersed enough to be fully covered, and then kept at a fixed temperature. These will be identified by the term "immersed", to indicate that there is no flesh exposed to the air and there is no presence of a waterline. Though roughly equivalent to a "shallow" designation, the presence of a fixed temperature variable necessitates a delineation. "Immersed" will also apply to cases in which a depth is not indicated, but it is known that the body remained submerged until it was recovered. For terrestrial settings, this is much simpler. Mant (1987, 69) indicates that "below a certain depth the temperature will remain constant at a low temperature." In Hau et al. (2014), shallow is defined as any depth under 1.2 meters, thus that designation will be employed here for terrestrial cases.

The categories adipocere coverage, texture, color, and odor are the most variable and difficult to track systematically. This is because, unlike typical decomposition processes, adipocere formation does not have its own qualitative, macroscopic scoring system (Alfsdotter and Petaros 2021; Magni et al. 2021). Quantitative techniques, such as GC-MS (Magni et al. 2021), are not widely applicable in a forensic setting due to fiscal and time constraints. As discussed in the previous chapter, these variables can differ significantly depending on the mineral composition, such as sodium versus potassium ion concentrations (Gill-King 1997) and pH of the environment. Their inclusion here represents an initial attempt to standardize adipocere descriptions, with the hope that, as more cases are added to the database, a pattern will emerge that correlates macroscopic adipocere observations with the post-mortem interval, when incorporated with other environmental variables. Though hardly exhaustive, the descriptors are pulled directly and extrapolated from the cases collected in this study. Eventually, it may be useful to also incorporate mineral concentrations (e.g. sodium, potassium, magnesium, calcium per Moses 2012), but for the sake of brevity they will be excluded from the experiments conducted here.

The variable "adipocere odor" is especially difficult to define. Though there are several descriptors found throughout the literature, only the smells that are distinct to adipocere (as opposed to decomposition broadly) are included. Though certain conditions (e.g. waterlogged) are associated with an "offensive odor" (Moses 2012, 590) this is hardly unique to adipocere. Further, the classification of "none" is used when a paper specifically indicates that the adipocere in question has no discernable odor.

"Fatty acid concentration" is also highly variable and difficult to simplify on a large scale. Work by Forbes et al. (2004) found that the relative fatty acid concentrations for a sample of adipocere correlate to stage of formation (i.e. early, intermediate, advanced). While stage of formation cannot be tracked to an exact PMI, when combined with other environmental factors, it can ideally contribute to a more precise PMI estimate. While most studies that analyze fatty acid concentration using GC-MS incorporate a set range of saturated and unsaturated fatty acids, this database will only track the fatty acids that seem particularly indicative of stage of formation according to Forbes et al. (2004). In early stages of formation, oleic acid (unsaturated) will decrease as palmitic acid (saturated) increases. During the intermediate stage, stearic acid (saturated) will increase as oleic acid continues to decrease. Also, palmitoleic and linoleic acids (unsaturated) should be "almost completely degraded" (Forbes et al. 2004, 569). Advanced stage adipocere formation has generally high saturated fatty acid concentrations, with little to no unsaturated fatty acid concentrations (oleic, palmitoleic, or linoleic acids). Palmitic acid "should represent more than half the total fatty acid composition" (Forbes et al. 2004, 570). Stage of formation as determined by the total percentage of saturated fatty acids for an adipocere sample comes from Forbes et al. (2004), Notter et al. (2009), and Notter and Stuart (2012). The threshold values for palmitoleic and linoleic acid were established from the values in the studies analyzed for this paper, as well as the information provided from the literature indicating that they will be virtually absent from advanced stage adipocere. Stearic acid, though noted as an indicator of stage of formation, undergoes β-oxidation to convert to palmitic acid, and thus will increase in the intermediate stage, but decrease in later stages (Forbes et al. 2004). This makes it difficult to determine if a low concentration is due to an early stage of adipocere formation, or a later stage, unless the palmitic acid concentration is also considered. Thus, the database will only include palmitic acid concentration.

To test the precision of the PMI estimates generated against actual cases, a total of six studies were identified and theoretically "plugged" into the database's "search engine" in an experimental manner. Studies were chosen based on the following criteria: they were not already part of the database, they provided enough information to represent several variables in the database, and they recounted a study of a freshwater, terrestrial shallow burial, or marine context. Due to limited availability, the second terrestrial shallow burial experiment (Hill and Pokines 2022) used an experimental study for reference, as opposed to a case study. The specific factors of each test study article were assigned to a database variable selection either directly or via extrapolation (Table 3), then experimental runs were conducted to test how the database would operate with a real-world case. The results of these runs are outlined in the Results section.

3.2.2 Inputting Variables

At present, there is no set method for applying the database to a case of unknown PMI. Ideally, users will identify all variables relevant to adipocere formation in their case and choose the most similar selections from the variables in Table 2. For an example of this process, please see Tables 4 and 5. Of note, as variables are filtered in the database, other variable categories will be reduced. For instance, once context is filtered to only include rivers and lakes, remaining samples may only report cold water cases, even if warm freshwater cases are generally represented in the database. This means that if the most accurate variable selection is not represented in the pool of samples, the user will need to identify the best alternative selection. In the example described above, this may mean filtering out cold samples, but leaving samples with an unknown temperature variable (i.e. Blanks).

3.2.3 Predictions

Due to the limitations of the database – in particular the frequency at which certain variables such as adipocere texture, color, and odor were omitted from sources – it is predicted that the database will produce accurate, but not exact, PMI estimates. Accurate is defined here as encompassing the known PMI, whereas exact refers to an estimate that is identical, or nearly identical, to the known PMI. For cases with known PMI on the order of years as opposed to days or months, the database may severely underestimate the PMI, especially for freshwater cases because the database does not contain any known PMIs that exceed 18 months. Furthermore, because warm context cases are notably absent from the database, especially for aquatic categories, it is predicted that the database will fail to produce an exact PMI estimate for any cases that take place in overtly warm or tropical environments.

4 RESULTS

Table 3 demonstrates how the variables of the test studies were translated into database variables for each experimental run. Runs were terminated when all applicable variables had been refined, or when only one or two samples were left in the database. The term "unrefined" indicates that a decision was made to not further narrow down a variable category. This was often due to other variables being deemed more important in influencing PMI formation. For instance, refining by U.S. state, subject (i.e. human versus porcine), or sex, variables that were not refined for any of the runs, would have meant subsequently limiting the number of available selections for such variables as temperature, pH, clothing, or adipocere coverage. The term "unreported" indicates that a variable category was not refined because it was not reported in the test study. Of note, just because a variable was not reported in the test study does not necessarily mean it was not refined in a run.

Furthermore, within the Excel database, each variable has an automatically generated list of selection options based on the associated entries; if there is no entry in a cell, the selection is referred to as "blank". The use of the term "Blanks" in Table 3 indicates that a variable was refined with this selection, and generally indicates that a selection was excluded, but that the absence or unknown presence of a variable was not deemed sufficiently influential to the PMI estimate output. This will be discussed further in the next section. Lastly, often a variable was unrefined because it was not reported in any of the remaining database samples after another variable was refined. The phrase "unreported/refined for any remaining samples" is meant to indicate that the decision to not refine a variable was due to its absence in the database, and not a deliberate decision on the part of this paper's author.

Table 4 Source Articles and Experimental Runs

Source – Ecosystem Category - Experiment	Article Variables	Database Variable Selections – Run 1	Database Variable Selections – Run 2	Database Variable Selections – Run 3
Cotton et al. (1986) – Freshwater Submersion – Experiment A	Duluth, Minnesota harbor Two human bodies, adult female and male Found in a damaged, flattened automobile (unknown when it was damaged). Parts of hands and feet missing Extensive subcutaneous adipocere formation: face, neck, trunk, extremities (absent from peritoneal and thoracic cavities) No decomposition odor. Extensive temperature readings for the past 5 years during immersion; at the time of immersion, water temperature at its highest point during the year. About 21°C when bodies went into the water, then cooler for the rest of the year and cycled this way for 5 years, with the temperature never dipping below 0°C. Depth not indicated but submerged for the entire 5 years. **PMI = 5 years almost to the date**.	• Ecosystem – “Freshwater” • Context – “Lake”, “River”, and “Irrigation Channel”. • Temperature – “Cool” (9-20°C). • US State – unrefined. • Soil – unreported for remaining samples. • Clothing – unrefined/unreported. • Fibers – unreported. • Additional Coverage – Blanks (excluded *n* = 1 “Blanket; Sheet”) • Depth – unrefined. • pH – unreported. • Subject – unrefined. • Sex – unrefined. • Adipocere coverage – Blanks (excluded *n* = 1 “Partial”) • Adipocere Texture, Color, and Odor – unreported. • Total Saturated Fatty Acid – “Advanced”. • Other Chemical Analysis – unrefined/unreported.	• Ecosystem – “Freshwater”. • Context – “River”, “Pond”, “Lake”, “Sinkhole”, “Irrigation Channel” (excluded Blanks, “Stream”, and “Marshy Pond”). • Temperature – excluded “Cold” but included Blanks and “Cool”. • US State – unrefined. • Soil – unreported. • Clothing – unreported/unrefined. • Fibers – unreported. • Additional Coverage – excluded “Blanket; Sheet”. Included Blanks and “Sunken Car”. • Depth –Blanks, “Shallow” and “Deep” (excluded “Surface”). • pH – unreported. • Subject – unrefined. • Sex – unrefined. • Adipocere coverage – excluded “Partial”, included Blanks and “Extensive”. • Adipocere Texture, Color, and Odor – unreported. • Total Saturated Fatty Acids – excluded “Intermediate”, included “Advanced” and Blanks.	Not run.

			• Other Chemical Analysis – unrefined/unreported.	
Sikary and Murty (2015) – Freshwater Submersion – Experiment B	Subtropical region Cases from May-October with average room temperature of 27-39°C with notable humidity. Fully clothed for all cases. Extensive adipocere formation for all bodies recovered from the water. 5 cases of drowning included in case study, all washed up on riverbanks: 1. 5-year-old female "Face, Front & back of trunk, both upper limbs, both lower limbs" (661) **PMI = 3 days and 5 hours**. 2. 55-year-old male "Front & back of trunk, both upper limbs except hands, both lower limbs except feet" (661) **PMI = 1 day and 4 hours**. 3. 38-year-old male "Abdomen, chest (not prominent), both arms (not prominent)" (661) **PMI = 2 days and 9 hours**. **4.** 14-year-old male "Face, Front & back of trunk, both upper limbs except hands, both lower limbs except feet" (661) **PMI = 1 week**. 5. 35-year-old male "Front of trunk, both upper limbs except hands, front of both lower limbs except feet" (661) **PMI = 3 days**.	• Ecosystem – "Freshwater". • Context – "River". • Temperature – No remaining samples with warm water. • All other variables unreported for remaining samples except chemical composition. • Total Saturated Fatty Acid – "Advanced. • Other Chemical Analysis – unreported/unrefined.	• Ecosystem – "Freshwater". • Context – unrefined. • Temperature – "Warm" and Blanks. • U.S. State – unrefined. • Soil – unrefined. • Clothing – "Fully Clothed". • One sample remaining – run terminated.	Not run.
Schotsmans et al. (2011) –	Coniferous Woodland in Belgium during Spring. Naked.	• Ecosystem – "Terrestrial".	• Ecosystem – "Terrestrial".	Not run.

Terrestrial Shallow Burial – Experiment C	Shallow burial (30-42 cm). Right leg and arm, and part of temporal bone exposed to the surface. Extensive adipocere covering (head, neck, right shoulder, upper torso, left leg) No odor. Right leg desiccated. Minimal scavenger and insect activity. Small, thin 65-year-old male. Wife of suspect confirmed victim had been killed around the time he went missing. Climate determined as "Northwest European Maritime climatic conditions found in Belgium". Sandy soil. pH 2.6-4. Temperature for 25-day window of internment: 5-24°C, with internment period going into the winter months. This averages 14.5°C during the internment period. **Went missing 7 months earlier.**	• Context – "Burial", "Burial, Woodland", and "Burial, Open-Woodland" (excluded "Damp Burial" and "Saturated Burial"). • Temperature – "Cool/Warm", and "Warm". • State - unrefined. • Soil - unrefined. • Clothing – Blanks, excluded "Partially Clothed". • Fibers - unrefined. • Additional Coverage – unrefined. • Depth – unrefined. • pH – unrefined. • Subject – unrefined. • Sex – unrefined. • Adipocere Coverage – unrefined. • Adipocere Texture, Color, and Odor – unreported for remaining samples. • Other Chemical Analysis – unreported/unrefined.	• Context – "Burial, Woodland", and "Burial, Open-Woodland" (excluded "Damp Burial", "Saturated Burial", and "Burial"). • Temperature – "Cool/Warm", and "Warm". • State - unrefined. • Soil – unrefined. • Clothing – Blanks, excluded "Partially Clothed". • Fibers - unrefined. • Additional Coverage - unrefined. • Depth – unrefined. • pH – unrefined. • Subject – unrefined. • Sex – unrefined. • Adipocere Coverage, Texture, Color, and Odor -unreported for the remaining samples. • Chemical analysis – unreported.	
Hill and Pokines (2022) – Terrestrial Shallow Burial – Experiment D	Massachusetts Temperature - climate with low to moderate humidity and annual average temperature of 6.8-14.8°C. Need to account for temperatures from April 2020-March 2021 (since testing a sample collected after 12 months). The average of each month's average is 10.5°C with a low of -2.5°C and a high of 24.2°C. Mixed tree coverage with limited shade (2194). Pig remains.	• Ecosystem – "Terrestrial". • Context – "Burial", "Burial, Open wooded", "Burial, Woodland". • Temperature – unrefined. • U.S State – unrefined. • Soil – "Sand". • Clothing – unrefined. • Fibers – unrefined. • Additional Coverage – unrefined. • Depth – unrefined. • Subject – unrefined.	• Ecosystem – "Terrestrial". • Context – "Burial", "Burial, Open Wooded", "Burial, Woodland". • Temperature – unrefined. • U.S. State – unrefined. • Soil – "Sand" and Blanks (excluded *n* = 1 "Loamy Sand"). • Clothing – Blanks and "Naked".	• Ecosystem – "Terrestrial". • Context – "Burial", "Burial, Open-Wooded". • Temperature – unrefined. • Clothing – "Naked", Blanks. • Fibers – unrefined. • Additional Coverage –

	Sandy soil. pH 3.81-4.23 (3.99 average). for shallow horizon. pH 3.94-4.67 (4.22 average) For deep horizon. Burial at 30 cm (classified as shallow) and 60 cm (classified as deep). Plastic wrapped samples presented with more adipocere formation than unwrapped samples. Wrapped samples decomposed slower. Wrapping remains may obscure time of death estimates. "Rate of change" (2201) slower for the colder seasons of months 9-18. *Chose sample S12W2 (Shallow Wrapped sample 2, collected at month 12). **PMI = 12 months.**	• Sex, Adipocere Coverage, Texture, Color, Odor – unreported for remaining samples. • Oleic Acid – "Late". • Other Chemical Analysis – unreported/unrefined.	• Fibers – unreported for remaining samples. • Additional Coverage – "Plastic". • Two samples remaining – run terminated.	"Plastic", Blanks. • Depth – unrefined. • pH – unrefined. • Subject – unrefined. • Sex – unrefined. • Adipocere Coverage – "Extensive" (excluded *n* = 1 "Minimal/Lower Limbs"). • Adipocere Texture, Color, Odor – unrefined. • Chemical Analysis – unreported.
Dumser and Türkay (2008) – Marine Submersion – Experiment E	Mediterranean Sea. Fully clothed in flight suit, head bare. Partly hanging out of helicopter with head resting on sea floor. The occipital part of skull resting on sea floor had adipocere where the scalp tissue was still preserved. It also extended down to the neck. The rest of the skull was partly skeletonized. Some scavenger activity by decapods (e.g. crabs). Temperature = 13°C. Deep depth – 600 m (bathyal – lack of light). **PMI = 34 days.**	• Ecosystem – "Marine". • Context – "Ocean" (excluded *n* = 1 "Ocean/Beach"). • Temperature – "Cool". • U.S State – unrefined. • Soil – unrefined. • Clothing – "Partially Clothed" and Blanks. • Fibers – unreported for remaining samples. • Additional Coverage – Blanks (excluded *n* = 1 "Closed Cabin"). • Depth – "Immersed" and "Deep". • Subject – unrefined. • Sex – unrefined. • Adipocere Coverage – "Subcutaneous/Face/Abdomen", "Minimal, and Blanks (excluded *n* = 1 "Subcutaneous/Extensi	• Ecosystem – "Marine". • Context – "Ocean" (excluded *n* = 1 "Ocean/Beach"). • Temperature – "Cool" (10-14.55°C). • U.S State – unrefined. • Soil – unrefined. • Clothing – "Partially Clothed" and Blanks. • Fibers – unreported for remaining samples. • Additional Coverage – Blanks (excluded *n* = 1 "Closed Cabin"). • Depth – "Deep". • Subject – unrefined. • Sex – unrefined. • Adipocere Coverage – Blanks, "Minimal", "Subcutaneous/Face/	Not run.

		ve" and n = 1 "Cutaneous/Subcutaneous"). • Adipocere Texture, Color, and Odor – unreported. • Chemical Analysis – unreported.	Abdomen" (excluded n = 1 "Subcutaneous/Extensive" and n = 1 "Cutaneous/Subcutaneous"). • Adipocere Texture, Color, and Odor - unrefined. • Chemical Analysis – unreported.	
De Donno et al. (2014) – Marine Submersion – Experiment F	52 victims of a shipwreck in the Adriatic Sea. 800 m deep. Sandy and muddy sea floor. 28 females, 24 males, Found within holds of the ship ("sequestered environment"). 23 children (less than 15 years), with 43 of the individuals younger than 35 years. Ammoniacal odor. Extensive adipocere formation (Chest, abdominal wall, buttocks). 4°C for the whole 7 months. Fully clothed/heavy clothing. **PMI = 7 months.**	• Ecosystem – "Marine". • Context – "Ocean" (excluded n = 1 "Ocean/Beach"). • Temperature – Blanks and "Cool" (10-14.55°C). • U.S. State – unrefined. • Soil – unrefined. • Clothing – unrefined. • Fibers – unrefined. • Additional Coverage – unrefined. • Depth – "Deep", "Immersed". • pH – unreported. • Subject – unrefined. • Sex – unrefined. • Adipocere Coverage – "Extensive", "Subcutaneous/Face/Abdomen", "Subcutaneous/Extensive", "Cutaneous/Subcutaneous". • Adipocere Texture, Color, and Odor - unrefined. • Oleic Acid – Blanks (excluded n = 1 "Early"). • Other Chemical Analysis – unreported/unrefined.	• Ecosystem – "Marine". • Context – "Ocean". • Temperature – "Cool" (10-12°C). • U.S. State – unrefined. • Soil – unrefined. • Fibers – unrefined. • Additional Coverage – "Environment (ship cabin)". • One sample remaining – run terminated.	Not run.

*Tested one sample (S12W2) out of n = 56. This sample is the only one that was wrapped and had an adipocere coverage of greater than 25%.

Table 5 Abbreviated Variables for Experimental Runs

Test Study	Test Study Variables	Database Variables – Run 1	Database Variables - Run 2	Database Variables – Run 3
Experiment A – Cotton et al. (1986)	• Minnesota harbor • Two human bodies recovered – one male, one female • Recovered from a flattened, submerged automobile • Extensive subcutaneous adipocere formation: face, neck, trunk, extremities (absent from peritoneal and thoracic cavities) • No decomposition odor • 21°C when the bodies when into the water, and known temperatures of the water for the next 5 years • Bodies submerged for 5 years at unknown depth	• Ecosystem: Freshwater • Context: Lake, River, Irrigation Channel • Temperature: Cool (9-20°C) • Additional Coverage: Blanks • Adipocere Coverage: Blanks • Total Saturated Fatty Acid: Advanced	• Ecosystem: Freshwater • Context: River, Pond, Lake, Sinkhole, Irrigation Channel • Temperature: Blanks, Cool • Additional Coverage: Blanks, Sunken Car • Depth: Blanks, Shallow, Deep • Adipocere Coverage: Blanks, Extensive • Total Saturated Fatty Acids: Advanced, Blanks	
Experiment B – Sikary and Murty (2015)	• Subtropical region • Cases from May-October with average room temperature of 27-39°C with notable humidity. • Fully clothed for all cases. • Extensive adipocere formation for all bodies recovered from the water. • 5 cases of drowning included in case study, all washed up on riverbanks • 1) 5-year-old female "Face, Front & back of trunk, both upper limbs, both lower limbs" (661) • 2) 55-year-old male "Front & back of trunk, both upper limbs except hands, both lower limbs except feet" (661) • 3) 38-year-old male "Abdomen, chest (not prominent), both arms (not prominent)" (661) • 4) 14-year-old male "Face, Front & back of trunk, both upper limbs except hands, both lower limbs except feet" (661) • 5) 35-year-old male "Front of trunk, both upper limbs except hands, front of both lower limbs except feet" (661)	• Ecosystem: Freshwater • Context: River • Total Saturated Fatty Acids: Advanced	• Ecosystem: Freshwater • Temperature: Blanks, Warm • Clothing: Fully Clothed	

Experiment C – Schotsmans et al. (2011)	• Coniferous woodland in Belgium during Spring • Naked • Shallow Burial (30-42 cm) • Adipocere covering head, neck, right shoulder, upper torso, left leg • No odor • Small, thin 65-year-old male • Sandy soil • pH 2.6-4 • Temperature for 25-day window of internment: 5-24℃, with internment period going into the winter months. This averages 14.5℃ during the internment period	• Ecosystem: Terrestrial • Context: Burial, Burial (Woodland), Burial (Open-Woodland) • Temperature: Cool/Warm, Warm • Clothing: Blanks	• Ecosystem: Terrestrial • Context: Burial (Woodland), Burial (Open-Woodland) • Temperature: Cool/Warm, Warm • Clothing: Blanks	
Experiment D – Hill and Pokines (2022)	• Massachusetts • Temperature for internment period ranged from -2.5℃ to 24.2℃ with a mean of 10.5℃ • Mixed tree coverage • Used pig remains • Sandy soil • pH 3.81-4.23 for shallow horizon and 3.94-4.67 for deep horizon • Two burial depth: shallow (30 cm) and deep (60 cm) • Some samples wrapped in plastic • Used sample S12W2 which represented a shallow, wrapped body	• Ecosystem: Terrestrial • Context: Burial, Burial (Open Wooded), Burial (Woodland) • Soil: Sand • Oleic Acid: Late	• Ecosystem: Terrestrial • Context: Burial, Burial (Open Wooded), Burial (Woodland) • Soil: Sand, Blanks • Clothing: Naked, Blanks • Additional Coverage: Plastic	• Ecosystem: Terrestrial • Context: Burial, Burial (Open Wooded) • Clothing: Naked, Blanks • Additional Coverage: Plastic, Blanks • Adipocere Coverage: Extensive
Experiment E - Dumser and Türkay (2008)	• Mediterranean Sea • Fully clothed in flight suit with a bare head • Partly hanging out of helicopter with head resting on the sea floor • Adipocere on preserved soft tissue of scalp where head rested on the ocean floor • Adipocere extended to neck • Temperature of 13℃ • Depth of 600 m (deep)	• Ecosystem: Marine • Context: Ocean • Temperature: Cool • Clothing: Partially Clothed, Blanks • Additional Coverage: Blanks • Depth: Immersed, Deep	• Ecosystem: Marine • Context: Ocean • Temperature: Cool (10-14.55℃) • Clothing: Partially Clothed, Blanks • Additional Coverage: Blanks • Depth: Deep • Adipocere Coverage: Blanks, Minimal, Subcutaneous/Face/Abdomen	

		• Adipocere Coverage: Subcutaneous/ Face/Abdomen, Minimal, Blanks		
Experiment F - De Donno et al. (2014)	• 52 total human bodies from a shipwreck • Depth of 800 m • Found within the hold of a ship • Sandy and muddy seaf floor • 28 females, 24 males • 23 children victims, and 43 victims under 35 years of age • Ammoniacal odor • Adipocere formation extensive over check, abdominal wall, and buttocks • Temperature 4℃ • Fully clothed in heavy clothing	• Ecosystem: Marine • Context: Ocean • Temperature: Blanks, Cool (10-14.55℃) • Depth: Deep, Immersed • Adipocere Coverage: Extensive, Subcutaneous/ Face/Abdomen, Subcutaneous/ Extensive, Cutaneous/Sub cutaneous • Oleic Acid: Blanks	• Ecosystem: Marine • Context: Ocean • Temperature: Cool (10-12℃) • Additional Coverage: Environment (Ship Cabin)	

4.1 Freshwater Submersion Experiments

Experiment A (Cotton et al. 1986), run 1 (Figure 1) produced a minimum PMI estimate of 6-18 months (mean = 12 months) with *n* = 5. The known PMI is 5 years. The produced PMI estimate thus falls within the known PMI, though it underestimates the known PMI by 3.5 years. Experiment A, run 2 (Figure 2) produced a minimum PMI estimate of 3-18 months (mean = 10.5 months) with *n* = 8. This is a broader PMI range, and it is less than the known PMI of 5 years.

ID#	Ecoystem	Context	Temperature	Subject	TotalSatFattyAcid	OleicAcid	LinoleicAcid	PalmitoleicAcid	PalmiticAcid	MinPMI
E1	Freshwater	River	Cool (20°C)	Pig Adipose Tissue	Advanced (93%)	Late (7%)	Inter/Adv (0%)	Inter/Adv (<1%)	Advanced (54%)	6 months
E1	Freshwater	River	Cool (20°C)	Pig Adipose Tissue	Advanced (99%)	Late (1%)	Inter/Adv (0%)	Inter/Adv (0%)	Advanced (80%)	9 months
E1	Freshwater	River	Cool (20°C)	Pig Adipose Tissue	Advanced (99%)	Late (1%)	Inter/Adv (0%)	Inter/Adv (0%)	Advanced (75%)	12 months
E1	Freshwater	River	Cool (20°C)	Pig Adipose Tissue	Advanced (99%)	Late (1%)	Inter/Adv (0%)	Inter/Adv (0%)	Advanced (73%)	15 months
E1	Freshwater	River	Cool (20°C)	Pig Adipose Tissue	Advanced (100%)	Late (<1%)	Inter/Adv (0%)	Inter/Adv (0%)	Advanced (82%)	18 months

Figure 1 Freshwater Submersion Experiment A Run 1

ID#	Ecoystem	Context	Temperature	U.S. State	AdditionalCoverage	Depth	Subject	Sex	AdipocereCoverage	AdipocereTexture	AdipocereColor	TotalSatFattyAcid	OleicAcid	LinoleicAcid	PalmitoleicAcid	PalmiticAcid	MinPMI
E1	Freshwater	River	Cool (20°C)				Pig Adipose Tissue					Advanced (93%)	Late (7%)	Inter/Adv (0%)	Inter/Adv (<1%)	Advanced (54%)	6 months
E1	Freshwater	River	Cool (20°C)				Pig Adipose Tissue					Advanced (99%)	Late (1%)	Inter/Adv (0%)	Inter/Adv (0%)	Advanced (80%)	9 months
E1	Freshwater	River	Cool (20°C)				Pig Adipose Tissue					Advanced (99%)	Late (1%)	Inter/Adv (0%)	Inter/Adv (0%)	Advanced (75%)	12 months
E1	Freshwater	River	Cool (20°C)				Pig Adipose Tissue					Advanced (99%)	Late (1%)	Inter/Adv (0%)	Inter/Adv (0%)	Advanced (73%)	15 months
E1	Freshwater	River	Cool (20°C)				Pig Adipose Tissue					Advanced (100%)	Late (<1%)	Inter/Adv (0%)	Inter/Adv (0%)	Advanced (82%)	18 months
E3	Freshwater	Lake	Cool (9°C*^{2})	International		Shallow (3 m)	Pig Adipose Tissue			Soft; Greasy	White						3 months
E3	Freshwater	Lake	Cool (9°C^{2})	International		Deep (9.1 m)	Pig Adipose Tissue			Hard; Crumbly	White						3 months
CS4	Freshwater	Pond			Sunken Car		49-year-old Human	Male	Extensive (Entire body)				Late (9.8%)	Inter/Adv (0.8%)	Early (4.8%)	Early/Inter (47.6%)	4 months

Figure 2 Freshwater Submersion Experiment A Run 2

Experiment B (Sikary and Murty 2015), run 1 (Figure 3) produced a minimum PMI estimate of 6-18 months (mean = 12 months) with *n* = 5. The known PMI is between 1 day and 4 hours and 1 week, with a mean of 80.4 hours (3 days and 8.4 hours). Run 1 thus produced an overestimation of the known PMI by over 6 months. Run 2 (Figure 4), however, produced a minimum PMI estimate of 3 days with *n* = 1. This is almost identical to the known PMI average of 3 days and 8.4 hours.

ID#	Ecoystem	Context	Temperature	Subject	TotalSatFattyAcid	OleicAcid	LinoleicAcid	PalmitoleicAcid	PalmiticAcid	MinPMI
E1	Freshwater	River	Cool (20°C)	Pig Adipose Tissue	Advanced (93%)	Late (7%)	Inter/Adv (0%)	Inter/Adv (<1%)	Advanced (54%)	6 months
E1	Freshwater	River	Cool (20°C)	Pig Adipose Tissue	Advanced (99%)	Late (1%)	Inter/Adv (0%)	Inter/Adv (0%)	Advanced (80%)	9 months
E1	Freshwater	River	Cool (20°C)	Pig Adipose Tissue	Advanced (99%)	Late (1%)	Inter/Adv (0%)	Inter/Adv (0%)	Advanced (75%)	12 months
E1	Freshwater	River	Cool (20°C)	Pig Adipose Tissue	Advanced (99%)	Late (1%)	Inter/Adv (0%)	Inter/Adv (0%)	Advanced (73%)	15 months
E1	Freshwater	River	Cool (20°C)	Pig Adipose Tissue	Advanced (100%)	Late (<1%)	Inter/Adv (0%)	Inter/Adv (0%)	Advanced (82%)	18 months

Figure 3 Freshwater Submersion Experiment B Run 1

ID#	Ecoystem	Context	Temperature	Clothing	Subject	Sex	AdipocereCoverage	AdipocereTexture	AdipocereColor	AdipocereOdor	MinPMI
CS2	Freshwater	Marshy Pond	Warm (air tem 30-35°C)	Fully Clothed (present over body)	35-year-old Human	Male	Abdomen (Front and back trunk); Upper and lower limbs (excluding hands and feet); Cheeks	Soft; Greasy	Yellowish-White	Ammonia	3 days

Figure 4 Freshwater Submersion Experiment B Run 2

4.2 Terrestrial Shallow Burial Experiments

Experiment C (Schotsmans et al. 2011), run 1 (Figure 5) produced a minimum PMI estimate of 5-13 months (mean = 9 months) with *n* = 7. The known PMI is 7 months. Therefore, run 1 captured the known PMI. Run 2 (Figure 6) produced a minimum PMI estimate of 5-13 months as well, with *n* = 6. This range also captures the known PMI.

ID#	Ecoystem	Context	Temperature	U.S. State	Soil	Depth	pH	Subject	AdipocereCoverage	OleicAcid	LinoleicAcid	PalmitoleicAcid	PalmiticAcid	MinPMI
E5	Terrestrial	Burial, Woodland	Cool/Warm (4.4-36°C)*[6]	International	Sand	Shallow		Whole Pig		Early (12.4%)	Inter/Adv (0%)	Inter/Adv (0.2%)	Early/Inter (46.6%)	5 months
E5	Terrestrial	Burial, Woodland	Cool/Warm (4.4-36°C)*[6]	International	Sand	Shallow		Whole Pig		Early (15.7%)	Inter/Adv (0%)	Inter/Adv (0%)	Advanced (54.7%)	8 months
E5	Terrestrial	Burial, Woodland	Cool/Warm (4.4-36°C)*[6]	International	Sand	Shallow		Whole Pig		Late (7.2%)	Inter/Adv (0%)	Inter/Adv (0%)	Advanced (55.9%)	8 months
E5	Terrestrial	Burial, Woodland	Cool/Warm (4.4-36°C)*[6]	International	Sand	Shallow		Whole Pig		Late (5.5%)	Inter/Adv (0%)	Inter/Adv (0%)	Advanced (60.8%)	8 months
E5	Terrestrial	Burial, Woodland	Cool/Warm (4.4-36°C)*[6]	International	Sand	Shallow		Whole Pig		Late (3.4%)	Inter/Adv (0%)	Inter/Adv 0%)	Advanced (58.4%)	13 months
E5	Terrestrial	Burial, Woodland	Cool/Warm (4.4-36°C)*[6]	International	Sand	Shallow		Whole Pig		Late (4.5%)	Inter/Adv (0%)	Inter/Adv (0%)	Advanced (60.8%)	6 months
E6	Terrestrial	Burial	Warm (22°C)		Loamy Sand	Covered	Acidic (5.2)	Pig Adipose Tissue	Extensive	Late (5.1%)		Inter/Adv (0%)	Advanced (58.9%)	12 months

Figure 5 Terrestrial Shallow Burial Experiment C Run 1

ID#	Ecoystem	Context	Temperature	U.S. State	Soil	Depth	Subject	OleicAcid	LinoleicAcid	PalmitoleicAcid	PalmiticAcid	MinPMI
E5	Terrestrial	Burial, Woodland	Cool/Warm (4.4-36°C)*[6]	International	Sand	Shallow	Whole Pig	Early (12.4%)	Inter/Adv (0%)	Inter/Adv (0.2%)	Early/Inter (46.6%)	5 months
E5	Terrestrial	Burial, Woodland	Cool/Warm (4.4-36°C)*[6]	International	Sand	Shallow	Whole Pig	Early (15.7%)	Inter/Adv (0%)	Inter/Adv (0%)	Advanced (54.7%)	8 months
E5	Terrestrial	Burial, Woodland	Cool/Warm (4.4-36°C)*[6]	International	Sand	Shallow	Whole Pig	Late (7.2%)	Inter/Adv (0%)	Inter/Adv (0%)	Advanced (55.9%)	8 months
E5	Terrestrial	Burial, Woodland	Cool/Warm (4.4-36°C)*[6]	International	Sand	Shallow	Whole Pig	Late (5.5%)	Inter/Adv (0%)	Inter/Adv (0%)	Advanced (60.8%)	8 months
E5	Terrestrial	Burial, Woodland	Cool/Warm (4.4-36°C)*[6]	International	Sand	Shallow	Whole Pig	Late (3.4%)	Inter/Adv (0%)	Inter/Adv 0%)	Advanced (58.4%)	13 months
E5	Terrestrial	Burial, Woodland	Cool/Warm (4.4-36°C)*[6]	International	Sand	Shallow	Whole Pig	Late (4.5%)	Inter/Adv (0%)	Inter/Adv (0%)	Advanced (60.8%)	6 months

Figure 6 Terrestrial Shallow Burial Experiment C Run 2

Experiment D (Hill and Pokines 2022), run 1 (Figure 7) produced a minimum PMI estimate of 6-13 months (mean = 9.5 months) with *n* = 4. The known PMI is 12 months. Therefore, run 1 captured the known PMI. Run 2 (Figure 8) produced a minimum PMI estimate of 12.96 months-4.5 years (mean = 2.79 years) with *n* = 2. Run 2 therefore almost captures the known PMI of 12 months exactly, though it is 0.96 months longer. Run 3 (Figure 9) produced a minimum PMI estimate of 12 months-4.5 years (mean = 2.75 years) with *n* = 6. The low end of the estimated range captured the known PMI exactly.

ID#	Ecoystem	Context	Temperature	U.S. State	Soil	Depth	Subject	OleicAcid	LinoleicAcid	PalmitoleicAcid	PalmiticAcid	MinPMI
E5	Terrestrial	Burial, Woodland	Cool/Warm (4.4-36°C)*[6]	International	Sand	Shallow	Whole Pig	Late (7.2%)	Inter/Adv (0%)	Inter/Adv (0%)	Advanced (55.9%)	8 months
E5	Terrestrial	Burial, Woodland	Cool/Warm (4.4-36°C)*[6]	International	Sand	Shallow	Whole Pig	Late (5.5%)	Inter/Adv (0%)	Inter/Adv (0%)	Advanced (60.8%)	8 months
E5	Terrestrial	Burial, Woodland	Cool/Warm (4.4-36°C)*[6]	International	Sand	Shallow	Whole Pig	Late (3.4%)	Inter/Adv (0%)	Inter/Adv 0%)	Advanced (58.4%)	13 months
E5	Terrestrial	Burial, Woodland	Cool/Warm (4.4-36°C)*[6]	International	Sand	Shallow	Whole Pig	Late (4.5%)	Inter/Adv (0%)	Inter/Adv (0%)	Advanced (60.8%)	6 months

Figure 7 Terrestrial Shallow Burial Experiment D Run 1

ID#	Ecoystem	Context	U.S. State	AdditionalCoverage	Depth	Subject	AdipocereCoverage	MinPMI
CS5	Terrestrial	Burial	Arizona	Plastic	Shallow (0.46 m)	Human	Extensive	12.96 months
CS5	Terrestrial	Burial	Louisiana	Plastic	Shallow (0.91 m)	Human	Extensive	4.5 years

Figure 8 Terrestrial Shallow Burial Experiment D Run 2

ID#	Ecoystem	Context	Temperature	U.S. State	Soil	Clothing	AdditionalCoverage	Depth	pH	Subject	AdipocereCoverage	OleicAcid	PalmitoleicAcid	PalmiticAcid	MinPMI
E6	Terrestrial	Burial	Warm (22°C)		Loamy Sand			Covered	Acidic (5.2)	Pig Adipose Tissue	Extensive	Late (5.1%)	Inter/Adv (0%)	Advanced (58.9%)	12 months
CS5	Terrestrial	Burial		Arizona			Plastic	Shallow (0.46 m)		Human	Extensive				12.96 months
CS5	Terrestrial	Burial		Florida		Naked		Shallow (0.46 m)		Human	Extensive				36 months
CS5	Terrestrial	Burial		Louisiana			Plastic	Shallow (0.91 m)		Human	Extensive				4.5 years
CS5	Terrestrial	Burial		Missouri				Shallow (0.46 m)		Human	Extensive				24 months
CS5	Terrestrial	Burial		Missouri				Shallow (0.46 m)		Human	Extensive				24 months

Figure 9 Terrestrial Shallow Burial Experiment D Run 3

4.3 Marine Submersion Experiments

Experiment E (Dumser and Türkay 2008), run 1 (Figure 10) produced a minimum PMI estimate of 38 days-18 months (mean = 9.65 months) with n = 8. The known PMI is 34 days. Run 1 overestimated the known PMI by only 4 days. Run 2 (Figure 11) produced a minimum PMI estimate of 38 days-433 days (~14.4 months) (mean = 7.85 months) with n = 3. Run 2, again, overestimated the known PMI by 4 days.

ID#	Ecoystem	Context	Temperature	U.S. State	Clothing	Depth	Subject	Sex	AdipocereCoverage	TotalSatFattyAcid	OleicAcid	LinoleicAcid	PalmitoleicAcid	PalmiticAcid	MinPMI
E1	Marine	Ocean	Cool (20°C)			Immersed	Pig Adipose Tissue			Early (55%)	Early (40%)	Early (3%)	Early (2%)	Early (23%)	6 months
E1	Marine	Ocean	Cool (20°C)			Immersed	Pig Adipose Tissue			Early (45%)	Early (51%)	Early (3%)	Early (1%)	Early (24%)	9 months
E1	Marine	Ocean	Cool (20°C)			Immersed	Pig Adipose Tissue			Early (40%)	Early (52%)	Early (7%)	Early (1%)	Early (3%)	12 months
E1	Marine	Ocean	Cool (20°C)			Immersed	Pig Adipose Tissue			Early (48%)	Early (52%)	Inter/Adv (0%)	Inter/Adv (<1%)	Early (29%)	15 months
E1	Marine	Ocean	Cool (20°C)			Immersed	Pig Adipose Tissue			Intermediate (70%)	Early (25%)	Early (5%)	Inter/Adv (0%)	Early (41%)	18 months
E9	Marine	Ocean	Cool (14.55°C*[5])	International		Deep (15.2 m)	Whole Pig								40+ days (5.7 weeks)
CS6	Marine	Ocean	Cool (10-12°C)	International	Partially Clothed	Deep (65 m)	Adult Human	Male	Subcutaneous/Face/Abdomen						38 days (5.4 weeks)
CS6	Marine	Ocean	Cool (10°C)	International	Partially Clothed	Deep (80 m)	Adult Human	Male	Minimal						433 days (14.4 months)

Figure 10 Marine Submersion Experiment E Run 1

ID#	Ecoystem	Context	Temperature	U.S. State	Clothing	Depth	Subject	Sex	AdipocereCoverage	MinPMI
E9	Marine	Ocean	Cool (14.55°C*[5])	International		Deep (15.2 m)	Whole Pig			40+ days (5.7 weeks)
CS6	Marine	Ocean	Cool (10-12°C)	International	Partially Clothed	Deep (65 m)	Adult Human	Male	Subcutaneous/Face/Abdomen	38 days (5.4 weeks)
CS6	Marine	Ocean	Cool (10°C)	International	Partially Clothed	Deep (80 m)	Adult Human	Male	Minimal	433 days (14.4 months)

Figure 11 Marine Submersion Experiment E Run 2

Experiment F (De Donno et al. 2014), run 1 (Figure 12) produced a minimum PMI estimate of 38 days-433 days (mean = 7.85 months) with n = 4. The known PMI is 7 months. Run 1 captured the known PMI, particularly when the mean estimate is considered. Run 2 (Figure 13) produced a minimum PMI estimate of 433 days (~14.4 months) with $n = 1$. Run 2, therefore, overestimated the known PMI of 7 months.

ID#	Ecoystem	Context	Temperature	U.S. State	Clothing	AdditionalCoverage	Depth	Subject	Sex	AdipocereCoverage	AdipocereTexture	AdipocereColor	MinPMI
CS6	Marine	Ocean	Cool (10-12°C)	International	Partially Clothed		Deep (65 m)	Adult Human	Male	Subcutaneous/Face/Abdomen			38 days (5.4 weeks)
CS6	Marine	Ocean	Cool (10-12°C)	International	Partially Clothed		Deep (65 m)	Adult Human	Male	Subcutaneous/Extensive			68 days (9.7 weeks)
CS6	Marine	Ocean	Cool (10-12°C)	International	Partially Clothed		Deep (65 m)	Adult Human	Male	Cutaneous/Subcutaneous	Crumbly	Yellowish-Whitish	109 days (3.6 months)
CS6	Marine	Ocean	Cool (10°C)	International	Partially Clothed	Closed Environment (Ship cabin)	Deep (80 m)	Adult Human	Male	Extensive	Crumbly		433 days (14.4 months)

Figure 12 Marine Submersion Experiment F Run 1

ID#	Ecoystem	Context	Temperature	U.S. State	Clothing	AdditionalCoverage	Depth	Subject	Sex	AdipocereCoverage	AdipocereTexture	MinPMI
CS6	Marine	Ocean	Cool (10°C)	International	Partially Clothed	Closed Environment (Ship cabin)	Deep (80 m)	Adult Human	Male	Extensive	Crumbly	433 days (14.4 months)

Figure 13 Marine Submersion Experiment F Run 2

Because the produced PMI is a minimum estimate, an underestimation of PMI is considered a successful run. Thus, 8 out of 13 runs successfully produced an accurate PMI. This value does not account for the two runs in which the PMI was overestimated by only 4 days, and the one run in which it was overestimated by 0.96 months. Furthermore, at least one run from each experiment produced an accurate PMI (Table 5), except for the one experiment (E) in which both runs overestimated the PMI by only 4 days.

Table 6 Known Versus Estimated PMI

Experiment	Run	Known PMI	Estimated Minimum PMI	Difference (Mean Estimated PMI minus Known PMI)
A – Cotton et al. (1986)	1	5 years	6-18 months	-4 years
	2	5 years	3-18 months	-4.13 years
B – Sikary and Murty (2015)	1	1 days 4 hours – 1 week (mean = 3 days 8.4 hours)	6-18 months	+11.88 months (based on mean known PMI)
	2	1 days 4 hours – 1 week (mean = 3 days 8.4 hours)	3 days	-8.4 hours (based on mean known PMI)
C – Schotsman et al. (2001)	1	7 months	5-13 months	+2 months
	2	7 months	5-13 months	+2 months
D – Hill and Pokines (2022)	1	12 months	6-13 months	-2.5 months

	2	12 months	12.96 months-4.5 years	+21.48 months
	3	12 months	12 months-4.5 years	+21 months
E – Dumser and Türkay (2008)	1	34 days	38 days-18 months	+255 days (~8.5 months)
	2	34 days	38 days-433 days	+201.5 days (~16.8 months)
F – De Donno et al. (2014)	1	7 months	38 days-433 days	+11.68 months
	2	7 months	433 days	+29.08 months (~2.42 years)

5 DISCUSSION

5.1 Freshwater Submersion Experiments

Overall, the data for the freshwater samples in the database did not consistently report on soil, clothing, fibers, additional coverage (though this may be due to absence in the test study), pH value, adipocere coverage, adipocere texture, adipocere color, adipocere odor, and chemical properties. Of these variables, pH, clothing, and adipocere descriptors are particularly significant when it comes to determining stage of adipocere formation in a freshwater context. This is because an acidic pH may slow down formation, whereas clothing may speed up adipocere formation, and a slimy versus crumbly adipocere texture may indicate initial or advanced formation, respectively. Soil is also relevant, particularly if the remains were embedded in a silt or sand matrix. Chemical composition is another variable that is helpful for determining stage of adipocere formation, as well as PMI when combined with other environmental factors, but time and budget constraints make it unlikely that medicolegal laboratories will conduct this kind of testing in the future.

The first case (Experiment A – Cotton et al. 1986) represents a situation in which a micro-climate was likely produced in the form of a submerged car. However, the damage to the vehicle at some point during the five years of submersion would have allowed water, bacteria, and potentially scavengers into the interior of the vehicle. The database only contained 1 sample (CS4) in which a submerged car was present in a freshwater context. Given that the context was a pond and the PMI in that case was 4 months, it was not necessarily applicable to the test study. Specifically, if context is designed to reflect bacterial population, a pond will have a different microbiome compared to a harbor, lake, or river. Cotton et al. (1986) is also a case of prolonged PMI, which makes it a particularly insightful test study. On the other hand, the test study did not report on depth of submersion, texture, or color of the adipocere, presence or absence of clothing on the bodies, pH of the water, soil type, or any chemical analysis of the adipocere. Both run 1 and run 2 produced results that significantly underestimated the known PMI of 5 years. However, though the precision of the result is not ideal, it may be noted that 18 months was the upper limit of the PMIs represented among all the freshwater cases in the database. It is unknown whether the produced PMI estimate would have been longer had a longer-term case been included in the database. Future additions to the database should take this into consideration.

Temperature was precisely documented for the five years in which the individuals were submerged in the Cotton et al. (1986) case, and the authors postulate that adipocere formation sped up during warm periods in the harbor and slowed or stopped during cold periods. For run 1, Context was refined to best capture a freshwater harbor environment, without limiting the output to a small sample size. Once ecosystem and context were refined, the only temperature selection remaining was Cool (9-20℃). It was judged that this range captured the periods in which adipocere formation would have occurred well enough to eliminate the need for further selection refining. The source indicates that the temperature of the harbor never dipped below 0℃, and the individuals would have entered the water when it was 21℃ (Cotton et al. 1986). Run 2 was designed to incorporate the sample with the sunken car to test whether this variable would help produce a more precise PMI, even though *a priori* analysis indicated that the context, temperature, and PMI of this case did not align with the test study. Run 2 produced an even less precise PMI estimate of 3-18 months, highlighting the multivariate nature of PMI estimation from adipocere formation. Overall, run 2 excluded fewer variable selections, only excluding stream and marshy pond from the context category, and including blanks in the temperature category, to name some examples. In this case, a less detailed input led to a less precise PMI estimate.

Experiment B (Sikary and Murty 2015) involved a test study that failed to report on specific water temperatures, depth of submersion, types of clothing fibers, texture, color, or odor of the adipocere, pH of the water, soil type, or chemical composition of the adipocere. However, the months in which the cases were found was stated, as well as the subtropical nature of the region (Sikary and Murty 2015, 660). This test study demonstrated whether the database would account for adipocere formation in warm water. Only one sample in the database presents a case of a warm environment, and the context for that case is a marshy pond. Subsequently, run 1 produced a PMI estimate of 6-18 months, which falls outside the range of the known PMIs by at least 5 months. In this run, once context was refined to best capture the river context in the test study, the one warm sample was no longer represented in the remaining samples. Run 2 refined less variables in an attempt to capture the warm sample in the PMI output. In run 2, context was not refined at all, and temperature was refined to include the one warm sample as well as two samples that did not report on temperature to broaden the sample size. Once clothing was refined to reflect the fact that all the individuals in the test study were fully clothed, the only sample left was the warm context. This produced a PMI estimate of 3 days, which almost exactly captured the mean known PMI of 3 days and 8.4 hours, despite so many of the other variables not matching the test study. This is interpreted as emphasizing the important role that temperature plays in adipocere formation, at least in an aquatic setting. It might also indicate that context is not an accurate substitute for bacterial population type and density. More testing is required to test this conclusion further.

5.2 Terrestrial Shallow Burial Experiments

Terrestrial shallow burial cases make up the majority of the database, representing more samples than the other two ecosystems combined. This is due, in large part, to CS5 (Manhein 1997) who provided 19 out of the 41 total samples. However, these authors fail to provide essential data, such as temperature values, soil typing, clothing presence or absence for all samples, detailed adipocere descriptions, or chemical analyses of the adipocere. This paper deliberately chose to study shallow burial cases as opposed to deep burials to test if temperature played a significant role in adipocere formation. Almost all the other sources in the database included temperature values and soil typing. However, they consistently failed to report on the presence or absence of clothing, fiber types, additional coverage, pH, sex, and adipocere descriptions. While the exclusion of clothing is more than likely due to the use of porcine remains in all but three of the other sources, and therefore their presumed absence, pH and adipocere descriptors should have been provided given that pH significantly influences rate of adipocere formation (Forbes et al. 2005b). This also underscores the importance of developing standardized methods for qualifying adipocere on remains.

Experiment C (Schotmans et al. 2011) is an excellent test study as it reports on context, temperature, clothing, burial depth, pH, and even the body composition of the victim. Furthermore, the suspect in the case was apprehended, so much of the pre-burial context is known compared to most cases. While the test study does not provide a detailed description of the adipocere that formed, both run 1 and run 2 produced precise PMI estimates, so it does not seem to have negatively impacted the study. Both runs resulted in a PMI estimate of 5-13 months (mean = 9 months). It is known that the victim went missing 7 months prior to the body being recovered, and therefore the PMI estimate indicates to investigators that the victim died around the time they went missing. Based on testimony from one of the suspects, the victim died almost immediately after going missing. This is corroborated by the PMI estimate produced from the database. Furthermore, an entomological assessment in the case provided a minimum PMI of 3 months, meaning that the PMI estimate based on adipocere formation captured the known PMI better than could be provided by insect activity.

In general, run 2 differed from run 1 in that it included more narrow selections. Of note, run 2 only included woodland and open-woodland contexts, whereas run 1 also included generalized burials. This narrowing of context also refined the soil selections in run 2, incidentally resulting in all remaining samples reporting sandy soil. This better matched the test study, which also reported sandy soil. The temperature selections for both runs included cool/warm and warm; this was based on the assertion by investigators that adipocere likely formed quickly after internment, and that the temperature window during the first 25 days of internment was 5-24℃ (Schotsmans et al. 2011). While this decision did not shed much light on the importance of the temperature variable in producing an accurate PMI estimate from the database, it more realistically reflected how variable selections would be modified to produce different results. In this case, deliberately refining context to only include woodland and open-woodland cases incidentally limited the available selections for soil type and adipocere coverage, which still produced an accurate PMI estimate. In a real-world situation, this might be interpreted as verifying the accuracy of a PMI estimate because an accurate estimate was still produced when similar selections were made.

Experiment D (Hill and Pokines 2022) is also the only experiment in which 3 runs were performed, as will be explained momentarily. This test study was designed to test if the database could account for additional coverings, in this case plastic trash bags. It also represents a slightly extended PMI of 12 months. The variables not reported were detailed adipocere descriptions (excluding a general percentage of adipocere coverage) and chemical composition of the produced adipocere. This is especially disappointing given that the test study was experimental and thus the only test study wherein a description of adipocere and chemical analyses could reasonably be expected. Lastly, it should be noted that this study used fetal pigs as their test subjects. Experiment B in this paper also presents a case of subadults in the form of a 5-year-old-female and a 14-year-old male. The age of the subjects did not seem to influence the precision of the PMI estimate for Experiment B, but one case is too small of a sample size to confidently assert that this is not an important factor to consider in future applications of the database, especially when the literature definitively indicates that adipocere more often forms on the bodies of children (Gill-King 1997; Ubelaker and Zarenko 2010; Teo et al. 2014; Hanganu et al. 2017).

Run 1 attempted to plug in the test study variables most accurately, and thus refined soil to only include sand. However, this significantly narrowed the selections available for the other variables. All other variables were not available for the remaining samples in the database except depth (only shallow), subject (only whole pig), and chemical composition. Though the test study did not provide a chemical analysis of the adipocere, given that 26-50% of the fetal pig specimen displayed adipocere formation, oleic acid percentage was refined to late stage (≥10%). Run 1 produced a minimum PMI estimate of 6-13 months (mean = 9.5 months), which captures the known PMI of 12 months. To test if factoring in the plastic covering would increase the precision of the PMI estimate, run 2 included sand and blanks in the soil variable selections. Refining additional coverings to plastic only, two samples were left, and the run was terminated. However, the lower limit of the resultant PMI estimate of 12.96 months-4.5 years comes very close to the known PMI. Despite this, it still overestimates by at least 1 month, and could potentially mislead investigators given the extended upper limit of 4.5 years. Run 3 mimicked run 2, but it refined the context to only burials and open-wooded burials, and it included both plastic and blanks in the additional coverage category. Both runs, however, seem to show that soil did not play a factor in producing a more accurate PMI estimate. Whether this is due to a lack of reportability in the database, or if soil itself did not significantly impact adipocere formation in this case, is unknown. Less restrictions on the additional coverage variable produced a larger sample size, and adipocere coverage was refined to exclude minimal/lower limbs coverage and thus only included extensive coverage. The resulting PMI estimate still had an extended upper limit of 4.5 years, but the lower limit of 12 months captured the known PMI exactly. While this is interpreted as a successful run, the imprecise upper limit is still problematic.

5.3 Marine Submersion Experiments

The marine entries in the database are overall comprehensive, though they lack some reporting on soil, clothing, fibers, additional coverage, pH, adipocere texture, adipocere descriptions, and chemical properties. CS4 (Adachi et al. 1997) in particular fails to indicate temperature, presence or absence of clothing, fibers, if there are additional coverings, pH values, and adipocere texture, color, or odor. The authors also only indicate superficial descriptions of adipocere coverage (i.e. partial and extensive). However, they do conduct chemical analysis on the adipocere, only leaving out total saturated fatty acid concentrations. Lastly, the database would be greatly improved by the addition of cold and warm marine water samples.

Experiment E (Dumser and Türkay 2008) effectively tests if the database can account for a short PMI in a marine ecosystem. Run 1 presented several challenging scenarios, such as how to account for the clothing of the individual since they were fully clothed, but their head was bare, and adipocere formed only on the bare parts of the occipital and neck. Due to this, it was decided that the clothing variables would include partially clothed and blank selections. Furthermore, the individual was found still strapped into their helicopter seat, but with part of their body resting on the sea floor. Therefore, a closed cabin case was excluded from the additional coverage category. Because of the difficulties associated with defining depth in aquatic environments, immersed and deep selections were included, but surface and shallow selections were excluded from the run. Finally, adipocere coverage was difficult to establish since it was relatively minimal, and there was not an extensive description of its subcutaneous extent nor any texture, color, or odor analysis. Ultimately, one sample with a subcutaneous/extensive designation was excluded, along with one cutaneous/subcutaneous case. The resulting PMI estimate for run 1 of 38 days-18 months came close to the known PMI of 34 days. While it technically overestimates the PMI by 4 days, this is a small variation when it comes to PMI estimates in general. It was also able to provide a relatively precise estimate for a test study in a deep-water context with notable scavenger activity. This further calls into question the effect of depth on adipocere formation in aquatic environments.

Run 2 tested if refining the temperature to better reflect the known water temperature of 13°C would produce a more precise PMI estimate. The temperature variable was refined to only include cool temperature samples within the range of 10-14.55°C. Clothing and additional coverage selections remained the same as run 1. Incidentally, after refining the other variables, the only samples left were classified as deep-water cases. After refining adipocere coverage to exclude subcutaneous/extensive and cutaneous/subcutaneous selections, the resultant PMI estimate was 38-433 days (~14.4 months). Though slightly more precise than run 1, it still overestimated the known PMI by 4 days. Run 2 also only produced a sample size of one. The extended PMI estimate when compared to the known PMI may be due to depth, as the “deep” sample individual in the database was found at 80 m, and the test study individual was found at 600 m. It may also be due to scavenger activity that limited the degree of adipocere formation that would have normally occurred under similar, less exposed conditions. A larger sample size is needed to test these hypotheses.

Experiment F (De Donno et al. 2014) is unique in that it analyzes a case of 52 victims of a shipwreck in the Adriatic Sea, of which 23 of the individuals are subadult (less than 15 years of age). This test study is also highly detailed, providing soil type, temperature, submersion depth, adipocere coverage and odor, and extent of clothing. Unfortunately, the database does not have any cold-water samples, and the test study reports a constant temperature of 4℃ for the whole PMI of 7 months. For run 1, it was decided that cool (10-14.55℃) water and blanks would be included. There are also no cases in the database that report fully clothed individuals, so the selections of partially clothed and blanks were not refined any further. Another unique aspect of this test study is that the victims were recovered from a “sequestered environment” (De Donno et al. 2014, 439) in the form of a ship’s hold. Though one sample in the database also reports a closed environment, it was decided for run 1 that the additional coverage variable would not be refined. Like in experiment E, depth was refined to only include deep and immersed samples, given the extreme depth of 800 m reported in the test study. Unfortunately, though the test study reports a highly diagnostic odor of ammonia, none of the database samples report a similar odor, and therefore it could not be factored into either run. Though the chemical composition of the adipocere was not supplied in the test study, the extensive adipocere formation on the shipwreck victims made it reasonable to refine the oleic acid variable by excluding one sample classified as early. The resultant PMI estimate for run 1 of 38 days-433 days (~14.4 months) captures the known PMI of 7 months, though not precisely. The presence of clothing is known to speed up adipocere formation (Mant and Furbank 1957; Mant 1987; Forbes et al. 2005b), so the absence of fully clothed samples in the database may explain the overestimation of the PMI in run 1. The underestimation may be explained by the lack of cold-water samples in the database since cold water slows adipocere formation and would thus raise the PMI estimate.

Run 2 was designed to capture the database sample with the closed cabin environment. Also, this run included a slightly refined temperature variable to only reflect cool-water samples in the 10-12℃ range. After refining for temperature and additional coverings, only one sample remained. The PMI estimate was 433 days (~14.4 months), and thus overestimated the known PMI. This indicates that simply having a similar micro-environment or climate does not necessitate a similar PMI. Multiple factors need to be considered when attempting to estimate PMI from adipocere formation.

5.4 Summary

A severe limiting factor to this study has been the small sample size of the database used. The sample size for the output of each experimental run ranged from only 1-8. Furthermore, with such small samples, it is difficult to quantify what is considered a "successful" PMI estimate. In this study, a run was generally considered successful if it captured the known PMI. Future use of the database would ideally produce samples large enough to conduct statistical testing to determine standard deviations for a given output, as well as to quantify the accuracy of the database. At present, there appears to be no discernable pattern to the PMI estimate ranges; for some runs, the lower limit of the estimate is closer to the known PMI, for other runs it is the mean or upper limit of the estimate, with some overlap between the categories. A larger sample size will only result if reportability of pertinent variables in adipocere formation increases in both experimental and case studies.

This study is limited by the dearth of information often collected in experiments and during forensic cases. Given the vast array of variables that contribute to adipocere formation, it is essential that as much information as possible is recorded to create a comprehensive database. Therefore, the goals of this paper have been twofold: first, to test whether an adipocere database and search engine is realistic and produces precise results and second, to propose a modification of current perspectives and procedures surrounding adipocere analysis and discovery.

Even in experimental studies, extremely pertinent information is unreported. Most significantly is the exclusion of soil typing when reporting adipocere formation in terrestrial environments. Work by Forbes et al. (2005a) has shown that soil type can significantly impact the speed of adipocere formation (and thus stage of formation). Furthermore, temperature is, shockingly, not always reported in experimental and case studies, even though it is one of the most influential determinants of whether adipocere forms and how quickly. Though not as relevant for deep burials, where the soil will act as a refrigerator beyond a certain depth, temperature is still highly pertinent to cases of shallow burial, as were discussed in this thesis.

6 CONCLUSION

This study tested the potential applicability and reliability of a search-engine-style database for determining the post-mortem interval in a forensic context. As expected, the database failed to precisely predict an extended PMI of 5 years for a freshwater case. However, the database was able to precisely estimate PMI for a tropical case involving an aquatic environment. Determining whether this indicates true reliability of the database in estimating PMI, or whether it is due to chance, will require a larger and more varied collation of data. Future test studies that provide a chemical profile of the adipocere would also help validate the selection variables established for the saturated and unsaturated fatty acids, as well as highlight other diagnostic components such as mineral or hydroxy fatty acid concentrations.

Overall, ~61% (8 out of 10) of the runs and ~83% (5 out of 6) of the experiments were considered successful in producing an accurate PMI. Given the experimental nature of the various runs, it is not surprising that only about two-thirds of the runs produced a minimum PMI estimate that captured the known value. Importantly, this paper has demonstrated the future value of such a database in determining PMI for cases with adipocere formation. This paper has also provided a point of departure for the standardization of adipocere scoring and reporting. The cases included in the database presented here – freshwater, terrestrial shallow burial, and marine – are just a portion of all possible forensic contexts. Adipocere formation for terrestrial surface depositions and deep burials, brackish and other non-marine saltwater contexts, and enclosed (i.e. domestic) settings are of forensic interest as well.

Future researchers should be aware of the multivariate nature of adipocere formation, and consequently strive to collect as much information as possible when assessing a forensic or experimental case involving adipocere. Temperature values, the specifics of clothing and other coverings, soil type in terrestrial and aquatic contexts, and adipocere descriptions should be included in every report. Where large datasets are analyzed, such as coroner cases, these variables are also important to note for each case or type of case involving adipocere. Also, more research into how adipocere degrades over time will help improve PMI estimates in cases of extended PMI. As practitioners and researchers become more aware of the utility of adipocere in determining the post-mortem interval, hopefully these considerations will be incorporated into research designs and analyses.

APPENDICES

Appendix A

Thesis%20Data%20Ex
cel.xlsx

Appendix B

Search%20Engine%2
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