

PAPER**ANTHROPOLOGY**

Marcella Widya,¹ M.Sc.; Colin Moffatt,¹ Ph.D.; and Tal Simmons,¹ Ph.D.

The Formation of Early Stage Adipocere in Submerged Remains: A Preliminary Experimental Study*

ABSTRACT: In some circumstances, the presence of adipocere may retard decomposition and complicate postmortem interval estimation. This article explores the correlation between Accumulated Degree Days (ADD) and early stage formation of adipocere. Sixty wild rabbit (*Oryctolagus cuniculus*) carcasses were used in this experiment; a control group ($N = 30$) deposited directly on the ground surface and an experimental group ($N = 30$) completely submersed in water in individual buckets. Data (water and inner body temperature, pH, and total body score) were collected every 100 ADD. Results indicated that early stage adipocere is correlated to ADD and that its formation on submersed remains is more likely to occur after 630 ADD. Skin sloughing promoted the formation of adipocere. No adipocere was formed on any of the control group rabbits. This study also highlights the fact that multiple factors influence adipocere formation and it is suggested that further research needs to be conducted into this area.

KEYWORDS: forensic science, forensic anthropology, taphonomy, adipocere, postmortem submersion interval, Accumulated Degree Days

Adipocere is a greasy, wax-like substance often present on decomposing bodies found in damp environments. Its name is derived from the Latin for fat (*adeps*) and wax (*cera*) and it has been studied from as early as the 18th century (1). Adipocere is the product of the hydrolysis of triglycerides, which are primarily found in adipose tissue, but also in the lipids of cell walls. The hydrolysis of triglycerides yields free fatty acids, components of adipocere, and unsaturated fatty acids may then undergo beta-oxidation, producing saturated fatty acids during adipocere formation. This process is facilitated by anaerobic bacteria, such as *Clostridium perfringens* and *Clostridium frigidicantes*, which are naturally present in the human body (2). Once formed, adipocere can persist over a long period of time, sometimes centuries, depending on the bacterial activity of the surrounding environment (2–4).

Adipocere has been found on remains recovered from various environments, such as submersion (5–9) and burial settings (3,10,11) and even on remains recovered from bogs (12) and wrapped up in plastic (13). Under what exact circumstances adipocere will form, however, still remains unknown, although it is thought to be affected by multiple factors, each of which must be present and within certain thresholds for the process to occur (14).

The majority of published literature on adipocere is anecdotal and based on case studies where chemical analysis of found adipocere is conducted (2,12,15–17). These studies all indicate that a moist environment is needed for adipocere to form. As the formation is initially a hydrolysis reaction, water is needed for this reaction to occur. This does not specifically imply that (partial) submersion is necessary; damp soil and humid air also promote

adipocere formation (12,14,18). Other factors that may influence adipocere formation are the presence of clothing (5,19), the chemical composition of the surrounding environment (18,20,21), and disturbance of the remains (22,23).

Another major factor implicated in the formation process is temperature. There are inconsistent reports of adipocere formation in aquatic environments in relation to temperature. Cotton et al. (24) reported that adipocere was formed in water with temperatures higher than 21°C. Payne and King (22) stated that adipocere was formed in a water temperature of 27°C. Various studies suggest that adipocere is more likely to form at temperatures between 21 and 45°C, as this is the optimum temperature range for the *C. perfringens* bacteria (25,26). However, both Sledzik and Micozzi (17) and O'Brien and Kuehner (27) found that adipocere also forms in colder water temperatures, 4 and 9°C, respectively. Forbes et al. (18) and Mellen et al. (28) conducted experiments comparing the formation in a cold environment to its formation in a warm environment and concluded that adipocere takes longer to form in colder temperatures.

A recent review by Ubelaker and Zarenko (29) summarizes all published findings on adipocere, including the chemical process involved, the circumstances of formation, and the effect of adipocere on decomposition. It is made clear that multiple processes are involved in the formation of adipocere and that temperature can affect the rate of formation. The review also underlines the gaps in knowledge in this area and states that, while a lot of new discoveries have been made over the last two centuries, there is still a great deal to learn about adipocere. In some circumstances the presence of adipocere may retard decomposition of the underlying tissue (19,30). This has the potential to make postmortem interval (PMI) estimation in such cases difficult. Limited research has been conducted to assess the influence of adipocere formation on the estimation of PMI or the impact of adipocere formation on decomposition rate and pattern in relation to Accumulated Degree

¹School of Forensic and Investigative Sciences, University of Central Lancashire, Maudland Building, Preston PR1 2HE, U.K.

*Presented at the 62nd Annual Meeting of the American Academy of Forensic Sciences, February 22–27, 2010, in Seattle, WA.

Received 5 July 2010; and in revised form 6 Jan. 2011; accepted 9 Jan. 2011.

Days (ADD) (5,31). The aim of this research was to gain a new understanding of the early stages of adipocere formation in relation to ADD in submersed remains and to provide information that can assist in a more accurate estimation of PMI.

Materials and Methods

The experiment was conducted at TRACES (Taphonomic Research in Anthropology: Centre for Experimental Studies), 13 acres of rough pasture site with sparse covering of trees, in the northwest of England. Sixty wild rabbits (*Oryctolagus cuniculus*) killed in the annual cull were collected from a game dealer. This licensed facility is controlled by an Official Veterinarian and the research was approved by the UCLAN Animal Projects Committee. The rabbits, which had been killed within the previous 48 h, were stored at 4°C and appeared fresh when collected. All rabbits were weighed by making a small incision in a hind leg for use with a hanging scale (Super Samson; Salter Brecknell, Smethwick, West Midlands, U.K.). A surface control group ($N = 30$), protected with chicken-wire cages held down with tent pegs to prevent scavenging, was positioned in direct contact with the ground.

The rabbits of the experimental group ($N = 30$) were submersed in fresh tap water in individual plastic buckets, which were partially inserted into shallow holes in the ground for stabilization. Tap water was utilized for practical reasons of transport and was not meant to replicate a naturally occurring scenario other than that of corpses discovered in bathtubs after lengthy immersion (e.g., cases in the U.K. of up to a month). Chicken-wire fencing on top of the buckets was used to prevent carcass flotation and to ensure complete submersion throughout the duration of the experiment. Due to evaporation during the course of the experiment, some of the buckets required the addition of water to prevent incomplete submersion of the rabbits; care was taken not to disturb accumulation of materials on the water's surface. All buckets and control cages were distributed randomly on the TRACES field.

Type K thermocouples were placed in the abdomen of every third rabbit in both groups and in the water of every third bucket in the experimental group. Self-contained dataloggers were placed inside the last three control rabbits and in the water of the last six experimental buckets to measure and log temperatures continuously. The last six experimental rabbits had thermocouples (connected to dataloggers) inserted in their abdomen. One self-contained datalogger was placed on the ground surface near the rabbits to measure and record the ambient temperature at the site throughout the experiment.

Data Collection

The time frame for the experiment was preset for a fixed interval consistent with availability of resources, and ran from 16 May to 15 July 2009. Data were collected *c.* every 100 ADD from submersed rabbits and every 30 ADD from the control rabbits on the ground surface. The control group sampling period was based on Vass et al. (32) weight correction data for terrestrial decomposition and the findings of Adlam and Simmons (33), which suggested that the rabbits were predicted to complete the wet decomposition phase by 214 ADD. At every data collection point, three rabbits of each group were examined and discarded, except for the last discarding point at which six rabbits were discarded, due to lower than expected seasonal temperatures extending the data collection interval beyond a predetermined end date. Decomposition was recorded by visual examination of the carcasses in both groups. Three

regions of each rabbit carcass were visually examined: the head and neck, the trunk, and the limbs. These zones were scored separately, based on the body scoring systems created by Heaton et al. (34) and Adlam and Simmons (33) for the sample and control group, respectively. Particular attention was paid to the visual assessment of adipocere formation. The examined carcasses were cut open and subcutaneous tissue and that around the internal organs was examined for the presence of adipocere. Although this substance was not analyzed chemically, its unique appearance was consistent with early stage adipocere formation. Early adipocere has white/gray appearance and, while it is wet and slimy, it is a rather a waxy, cream-like paste in the process of thickening and unlike a runny liquid one would expect from liquefied adipose tissue, which would have a diluted, watery feel to it. Thus, we report what we perceived to be adipocere and the reader should be mindful of this definition throughout this article. Water and inner body temperatures were measured using a thermocouple reader and recorded in degree Celsius. Samples of soil from underneath the control rabbits and water from the experimental buckets were also taken to measure pH. Any changes in the water surrounding the rabbits (e.g., color, insect activity, algae growth) were visually observed and noted.

ADD Calculation

During the experiment, additional weather information was obtained from the Environmental Protection Weather Station, located *c.* 1.9 miles away. The weather station data were used to produce an approximate ADD scale used for sampling. After the conclusion of the experiment, temperature data from the ambient and water temperature dataloggers, for the control and submerged group, respectively, were used to recalculate a more accurate ADD used in the analyses. The calculated ADD is the PMI.

pH Measurement

Soil and water pH were measured using a Hanna Instruments pH Checker (Leighton Buzzard, U.K.), which was calibrated using standard pH buffers (pH 4, 7, 10) prior to sample measurements. Distilled water was added to the soil collected from underneath the control rabbits (10:25 w/v), and mixed thoroughly. The mixture was then left to settle and filtered through muslin. The pH of the filtrate was measured. The pH of the water from the experimental buckets was measured directly.

Insect Rearing

Maggots were collected from several rabbit carcasses of the control group and reared in plastic containers containing pork liver and saw dust to provide nutrition and cover. The reared adult insects were identified to species.

Statistical Analysis

All statistical analyses were conducted using the statistical software package R (v.R-2.8.0) (35). A comparison of decomposition score for the two treatment groups was analyzed by an additive model using the mgcv package (36) as a linear model was not a good fit of the data, even after transformation. A logistic regression model was used to analyze the proportions of rabbits developing adipocere in relation to ADD: as no distinction was made between adipocere phases, the data were binomial.

A repeated measures linear model (37) was used to compare the internal temperatures of the treatment groups with ADD.

Results

Total Body Score

The rabbits in the control groups started to show signs of bloating from 31 ADD onward. Oviposition was detected around 106 ADD and maggots were present at 139 ADD, nearly simultaneously with the first noted occurrence of fur loss. Maggots present on some of the carcasses did not migrate away to pupate, but rather used the remaining skin and fur as protection. Complete skeletonization was observed at 236 ADD.

The decomposition rate of the experimental rabbits was slower than that of the control groups ($t = 8.16$, $p < 0.001$, d.f. = 56.48, 90.1% deviance explained). Bloating started around 191 ADD and signs of fur slippage at the limbs, head, and neck area also occurred at this stage. Bone exposure was first noted at 534 ADD at the limbs, and disarticulation of the feet occurred at 582 ADD. Bone exposure at the trunk was observed at 680 ADD; however, at 755 ADD a single rabbit still exhibited intact skin and a bloated abdomen. At this final discarding point, only one carcass was fully skeletonized and disarticulated. The experiment was terminated at this juncture due to time constraints of the predetermined 2-month interval discussed earlier.

Adipocere

Substances consistent with the appearance of adipocere were absent on all of the rabbits of the control group, but were present on the rabbits of the experimental group at the later stages of the experiment. At 582 ADD a slimy, soft, white substance consistent with early stage formation adipocere was found on the hind limbs of two of the three experimental rabbits (Fig. 1). At subsequent discarding points, adipocere was present on the majority of the rabbits. Most of this was located around the lower region of the rabbit carcasses. However, white, slimy, fatty solids were also present in the surrounding water and were deposited on the rabbits themselves. The exact origin of this early adipocere was difficult to determine. It was observed that while the adipoceros rabbits showed signs of skin sloughing, the nonadipoceros rabbits did not and had intact skin. In

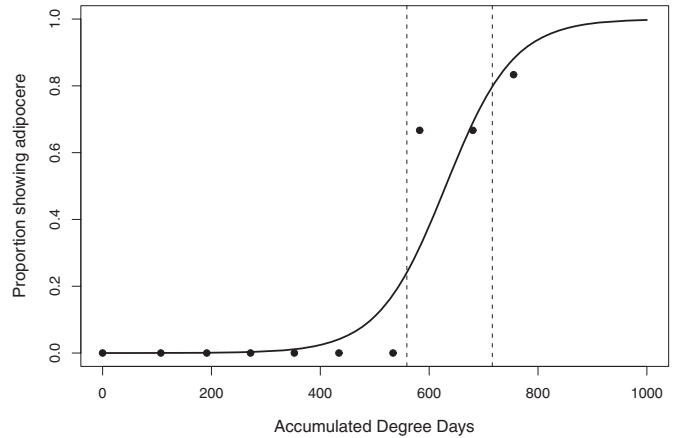


FIG. 2—Graph of percentage of rabbits with adipocere against ADD. The broken lines represent the ADD 95% confidence interval for 50% adipocere.

addition, the formation of adipocere was not detected on any of the internal organs. All such adipocere encountered had the same soft and slimy appearance and no visual distinction could be made at various ADD intervals; therefore, it was considered to be in the same phase of formation.

The logistic regression model was a good fit of the data (residual deviance = 3.17 on 8 d.f., $\chi^2 = 0.924$, $p = 0.923$), as Fig. 2 shows. A prediction of adipocere probability was produced from the model to give an ADD value, which for 50% adipocere was 631 ADD. A bootstrap approach was then used by randomly selecting (with replication) data points from each experimental ADD, and rerunning the model to produce 1000 predictions of ADD. The percentile method was then used to produce a 95% confidence interval for the prediction, which for 50% adipocere was from 559 to 716 ADD (superimposed on Fig. 2). Using the same approach, the estimate for 90% adipocere was 768 ADD with a 95% interval from 561 to 911 ADD, and for 95% adipocere, the estimate was 815 ADD with a 95% interval from 588 to 961 ADD. It is likely more accurate estimates would have been produced from a model based upon data representing higher proportions of adipocere, that is, from an experiment conducted over a longer period.



FIG. 1—Early stage adipocere on a rabbit carcass at 755 ADD.

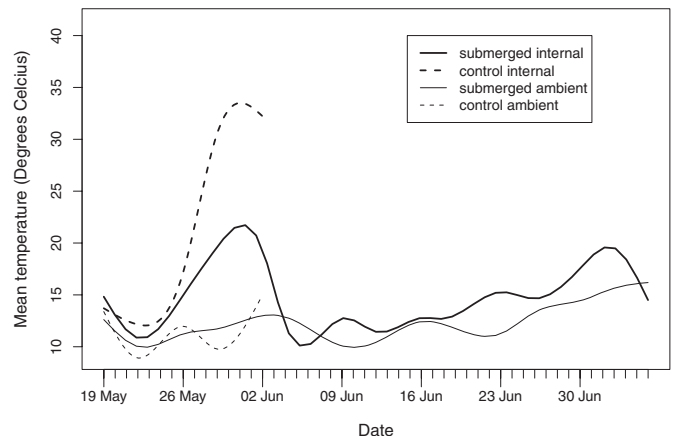


FIG. 3—Internal temperatures against date for treatment groups. Lines are smoothed splines of means.

Inner Body Temperatures

The inner body (abdominal) temperatures of both groups were initially similar (Fig. 3), however, around 24 May (c. 80 ADD), both began to rise, the control group more rapidly. The control group's temperatures continued to rise for another week, peaking at around 17°C above the ambient air temperature, soon before the end of wet decomposition and skeletal exposure for this group. The submerged group temperatures peaked around the same time, but the difference from ambient water temperature was only 8°C. Across the whole of the experimental period, the internal temperatures of the control group were significantly higher ($t = 5.48$, $p < 0.0001$, d.f. = 111), by around 5.2°C.

Water

An oily layer started forming on top of the buckets at 106 ADD, which thickened into a white layer over time. This layer, when broken up, formed solid conglomerates. The water in all buckets started out as transparent, but became darker and murkier during the course of the experiment. In several buckets, the water turned an opaque orange-brown color.

Algal growth was observed in nine of the buckets with the earliest observation at 434 ADD. The algae covered the sides of the buckets and in some cases the rabbit carcasses.

Insect Activity

The majority of the maggots present on the control carcasses were identified (by CM) as *Calliphora vomitoria*, *Calliphora vicina*, and *Protophormia terraenovae*. These species were not found on any of the rabbits of the experimental groups. However, from 434 ADD onward rat-tailed maggots were present in the majority of the buckets. Rat-tailed maggots are hoverfly (flower fly) larvae (Syrphidae: *Eristalis* spp.) (38,39).

pH

pH of the soil under the control samples was found to have a mean of 6.18 ± 1.22 (1 SD), but showed no pattern with time. The

pH of the water samples had a mean of 6.95 ± 0.44 , a significantly smaller variance than for the controls ($F_{33,14} = 6.6$, $p < 0.001$), initially decreasing then increasing from about 250 ADD. A full chemical and microbiological analysis of the water is presented in Table 1.

Discussion

Insect Activity

The much slower decomposition seen in the submerged carcasses can be attributed to an absence of feeding insects. Insect presence has been shown to be the most important factor governing decomposition rate after temperature (40,41). The only insects found in the buckets containing the submerged rabbits, rat-tailed maggots of *Eristalis* would not have affected decomposition as these larvae feed on particulate organic matter suspended in the water (38,39).

The greatest difference between the mean internal temperatures of the treatment groups was 14°C, which was on the 29 May (c. 140 ADD) and is best explained by maggot mass heat generation in the control group. Maggot masses are known to generate heat raising the mass temperature significantly higher than their environment (40,42,43). It is interesting to note that there was also a temperature rise in the submerged group despite the absence of necrophagous insects. A possible explanation is microbial heat generated during autolysis (44).

Adipocere

In the submerged rabbit carcasses, the likelihood of adipocere formation increased with time, or more accurately, ADD. If the experiment had been conducted over a longer period, adipocere formation was likely to have occurred in all such individuals. This supports the fact that adipocere formation is a feature related to the advanced stages of decomposition. Although the model presented here used rabbits, where adipocere formation became more likely than not around 630 ADD, it is suggested that a similar rate may be observable in carcasses of larger body mass (e.g., humans), as neither carcass size nor body fat content affects decomposition rate

TABLE 1—Chemical and microbiological analyses of the water throughout the experiment.

ADD		Chemical Analysis	ppm	Microbiological Analysis	CFU/mL		
0	Anions	Chloride (Cl ⁻)	7.0	Aerobic heterotrophic bacteria	1.8×10^4		
		Nitrate (NO ₃ ⁻)	1.98	Coliforms	20		
		Sulfate (SO ₄ ²⁻)	10.82	Anaerobic bacteria	0		
		Phosphate (PO ₄ ³⁻)	3.7				
	Cations	Sodium (Na ⁺)	6.0				
		Potassium (K ⁺)	0.53				
		Magnesium (Mg ²⁺)	1.15				
		Calcium (Ca ²⁺)	4.83				
		Iron (Fe)	0				
		Aluminium (Al)	0				
		Copper (Cu)	0				
		680	Not analyzed			Aerobic heterotrophic bacteria	1.0×10^{12}
						Coliforms	3.0×10^{11}
				Anaerobic bacteria	5.3×10^5		
961	Not analyzed			Aerobic heterotrophic bacteria	1.5×10^{14}		
				Coliforms	5.2×10^{13}		
				Anaerobic bacteria	2.2×10^7		

ADD, Accumulated Degree Days.

Chemical analyses reported in parts per million (PPM) and microbiological analyses reported in colony forming units per milliliter (CFU/mL). Anions were analyzed by Dionex ion-chromatography (Dionex (UK) Ltd., Camberley, Surrey, U.K.) and cations were analyzed by atomic absorption/emission. Yeasts and moulds were not isolated, likely due to their inability to compete with the bacteria.

in the absence of insects (41). Forbes et al. (45) discussed the formation of adipocere in varying animal species, concluding that, while it was directly related to the presence of sufficient adipose tissue, its formation rate, but not its fundamental composition, varied by taxon. As all rabbits utilized in this study presented with similar initial weights (1.3–1.9 kg), they can be presumed to have had similar fat content.

It was also observed that adipocere was only present on the rabbits on which skin slippage had occurred. This indicates that direct contact exposure of the adipose tissue to the water promotes adipocere formation. Skin slippage is one of many recognized features in decomposition which can be used to establish PMI (34). In cases where PMI estimation may have been complicated by adipocere formation, knowing that skin sloughing had to have occurred prior to when the adipocere could have been formed may assist in establishing a more accurate PMI.

O'Brien (14) mentioned the "Goldilocks" phenomenon, stating that the conditions had to be within optimum ranges (i.e., "just right") for adipocere formation to occur. Conditions affecting adipocere formation included the temperature, amount of moisture present, and pH. The variation at intermediate ADD seen in the experiment also suggests that there are more factors than merely temperature and insect exclusion need to exist for adipocere formation to occur.

The water surrounding the rabbit carcasses reached temperatures ranging from 6 to 26°C. This range is lower than the hypothesized ideal temperature range of 21 to 45°C (11,25), but still overlaps. It could be suggested that the temperatures were too low to stimulate the *C. perfringens* bacteria to reach their optimum activity, however, previous studies indicate that adipocere can be formed within the temperature range obtained in this experiment or even colder (17,27).

It was also suggested that if the environment was too wet, the tissue might liquefy and macerate before adipocere formation could occur (14). Although adipocere did indeed form, this could explain why the rabbits were in such an advanced decomposition stage, while the adipocere was still in its early formation phase. The rate at which the adipocere formed and the consistency of the early stage formation were not rapid enough to protect the tissue from the environment and the decomposition process. The tissues could have macerated before the adipocere could develop into the characteristic waxy, thicker paste.

However, as adipocere has known to reach a more advanced stage in fully submerged human remains (5–9), this cannot be the only factor influencing the results of this particular experiment. An explanation could be the fact that wild rabbits are fairly lean animals. As adipocere is more likely to form when lipids are present in abundance, it may be possible that the rabbit carcasses did not possess enough adipose tissue to facilitate advanced adipocere formation before liquefaction of tissues occurred in this aquatic environment.

The pH of the surrounding environment is also believed to affect adipocere formation. Even though the values were not precisely neutral for either group, the range is still considered to be intermediate, meaning the water was never too acidic or too basic for adipocere formation to be inhibited.

The type of water surrounding the carcass also affects the rate of adipocere formation on submersed remains. Adipocere has been observed to form in fresh (20,24), saline (8,20,46), and chlorinated water (20). A study by Yan et al. (20) found that formation of adipocere is slower in chlorinated and saline water, compared to tap water. This is due to the difference in chemical composition, affecting the bacterial activity. The presence of high concentrations of

electrolytes (as in saline) or bacteria destroying disinfectant (e.g., chlorinated water) inhibits the bacterial activity and therefore the adipocere production. As fresh tap water was used, this was not a factor in this study (see Table 1).

Two other factors that could have had an effect were the presence of algae and rat-tailed maggots in the water of the experimental buckets. The decomposing remains created a nutrient rich environment in the water from which both algae and larvae could feed (38,39,47). The presence of algae and insect larvae could potentially affect adipocere formation in several ways. Fatty acids and other chemical substances needed for adipocere formation may have been consumed by these organisms and hence adipocere formation diminished. It is also possible that the presence of the algae and insect larvae somehow suppressed bacterial activity and thus by extension the formation of adipocere. These suggestions are speculative and further research is required to resolve the issue.

Conclusion

The lack of advanced adipocere formation indicated that multiple factors are involved in the process of adipocere formation. Presence of water, an environment with a neutral pH, and temperatures that were within the optimal range were not sufficient to ensure complete adipocere formation. The effects of algae growth and presence of *Eristalis tenax* larvae could not be determined and may have confounded the formation of adipocere. Adipocere formation was only observed on remains in which skin sloughing had occurred. This suggests that exposure of adipose tissue to the water promotes adipocere formation and supports the suggestion that adipocere is a constituent of the later stage of decomposition. This feature could be used to assist in estimating a more accurate PMI when dealing with adipoceros remains.

The results of this study have demonstrated that early stage formation of adipocere is in response to an increase in ADD. The greater the ADD, the more likely adipocere is to form. It was also estimated that adipocere was more likely to form from 630 ADD onward under the conditions studied. This implies that remains presenting with adipocere would have had a minimum postmortem submersion interval of 630 ADD.

Acknowledgments

We thank Peter Cross and Rachel Cunliffe and the MSc Forensic Anthropology students of the 2008–2009 cohort for their support during all stages of this project. Our special thanks to Richard McCabe and L.H. Glynn Morton for their chemical and microbiological analyses of the water, respectively; any interpretations presented here are, however, solely our own. Thanks to Walker's Game for supplying the rabbits used in this experiment. Our thanks are also given to the two anonymous reviewers for their constructive and helpful comments.

References

1. Den Dooren de Jong LE, Dauvillier MS, Roman WB. On the formation of adipocere from fats. Contribution to the microbiology of systems containing two liquid phases. *Antonie Van Leeuwenhoek* 1961;27(1):337–61.
2. Fiedler S, Buegger F, Klaubert B, Zipp K, Dohrmann R, Wittmeyer M, et al. Adipocere withstands 1,600 years of fluctuating groundwater levels in soil. *J Archaeol Sci* 2009;36(7):1328–33.
3. Pfeiffer S, Milne S, Stevenson RM. The natural decomposition of adipocere. *J Forensic Sci* 1998;43(2):368–70.

4. Fründ HC, Schoenen D. Quantification of adipocere degradation with and without access to oxygen and to the living soil. *Forensic Sci Int* 2009;188:18–22.
5. Dix JD. Missouri's lakes and the disposal of homicide victims. *J Forensic Sci* 1987;32(3):806–9.
6. Lawler W. Bodies recovered from water: a personal approach and consideration of difficulties. *J Clin Pathol* 1992;45:654–9.
7. Inoue H, Iwasa M, Maeno Y, Koyama H, Sato Y, Matoba R. Detection of toluene in an adipoceratous body. *Forensic Sci Int* 1995;78:119–24.
8. Kahana T, Almog J, Levy J, Shmeltzer E, Spier Y, Hiss J. Marine taphonomy: adipocere formation in a series of bodies recovered from a single shipwreck. *J Forensic Sci* 1999;44(5):897–901.
9. Lucas J, Goldfeder LB, Gill JR. Bodies found in the waterways of New York City. *J Forensic Sci* 2002;47(1):137–41.
10. Ruttan RF, Marshall MJ. The composition of adipocere. *J Biol Chem* 1991;29(2):319–27.
11. Forbes SL, Stuart BH, Dent BB. The identification of adipocere in grave soils. *Forensic Sci Int* 2002;127:225–30.
12. Evershed RP. Chemical composition of a bog body adipocere. *Archaeometry* 1992;2:253–65.
13. Nushida H, Adachi J, Takeuchi A, Asano M, Yasuhiro U. Adipocere formation via hydrogenation of linoleic acid in a victim kept under dry concealment. *Forensic Sci Int* 2008;175:160–5.
14. O'Brien TG. Movement of bodies in Lake Ontario. In: Haglund WD, Sorg MH, editors. *Forensic taphonomy: the postmortem fate of human remains*. Boca Raton, FL: CRC Press, 1997;559–65.
15. Bass WM. Outdoor decomposition rates in Tennessee. In: Haglund WD, Sorg MH, editors. *Forensic taphonomy: the postmortem fate of human remains*. Boca Raton, FL: CRC Press, 1997;181–6.
16. Rodriguez WC. Decomposition of buried and submerged bodies. In: Haglund WD, Sorg MH, editors. *Forensic taphonomy: the postmortem fate of human remains*. Boca Raton, FL: CRC Press, 1997;459–68.
17. Sledzik PS, Micozzi MS. Autopsied, embalmed, and preserved human remains: distinguishing features in forensic and historic contexts. In: Haglund WD, Sorg MH, editors. *Forensic taphonomy: the postmortem fate of human remains*. Boca Raton, FL: CRC Press, 1997;483–92.
18. Forbes SL, Stuart BH, Dent BB. The effect of the burial environment on adipocere formation. *Forensic Sci Int* 2005;154(1):24–34.
19. Mant AK, Furbank R. Adipocere: a review. *J Forensic Med* 1957;4:18–35.
20. Yan F, McNally R, Kontanis EJ, Sadik OA. Preliminary quantitative investigation of postmortem adipocere formation. *J Forensic Sci* 2001;46(3):609–14.
21. Forbes SL, Dent BB, Stuart BH. The effect of soil type on adipocere formation. *Forensic Sci Int* 2005;154:35–43.
22. Payne JA, King EW. Insect succession and decomposition of pig carcasses in water. *J Georgia Entomol Soc* 1972;7:153–62.
23. Nawrocki SP, Pless JE, Hawley DA, Wagner SA. Fluvial transport of human crania. In: Haglund WD, Sorg MH, editors. *Forensic taphonomy: the postmortem fate of human remains*. Boca Raton, FL: CRC Press, 1997;529–52.
24. Cotton GE, Aufderheide AC, Goldschmidt VG. Preservation of human tissue immersed for five years in fresh water of known temperature. *J Forensic Sci* 1987;32:1125–30.
25. Tomita K. On putrefactions and floatations of dead bodies under water. *Hiroshima J Med Sci* 1976;24:117–52.
26. Corry JEL. Possible sources of ethanol ante- and post-mortem: its relationship to the biochemistry and microbiology of decomposition. *J Appl Bacteriol* 1978;44:1–56.
27. O'Brien TG, Kuehner AC. Waxing grave about adipocere: soft tissue change in an aquatic context. *J Forensic Sci* 2007;52(2):294–301.
28. Mellen PFM, Lowry MA, Micozzi MS. Experimental observations on adipocere formation. *J Forensic Sci* 1993;38(1):91–3.
29. Ubelaker DH, Zarenko KM. Adipocere: what is known after over two centuries of research. *Forensic Sci Int* 2011;208(1–3):167–72.
30. Šlaus M, Strinović D, Pečina-Šlaus N, Brikč H, Baličević D, Petrovečki V, et al. Identification and analysis of human remains recovered from wells from the 1991 War in Croatia. *Forensic Sci Int* 2007;171:37–43.
31. Forbes SL, Stuart BH, Dadour IR, Dent BB. A preliminary investigation of the stages of adipocere formation. *J Forensic Sci* 2004;49(3):566–74.
32. Vass AA, Bass WM, Wolt JD, Foss JE, Ammons JT. Time since death determinations of human cadavers. *J Forensic Sci* 1992;37(5):1236–53.
33. Adlam RE, Simmons T. The effect of repeated physical disturbance on soft tissue decomposition—are taphonomic studies an accurate reflection of decomposition? *J Forensic Sci* 2007;52(5):1007–14.
34. Heaton V, Lagden A, Moffatt C, Simmons T. Predicting the post-mortem submersion interval for human remains recovered from UK waterways. *J Forensic Sci* 2010;55(2):302–7.
35. R Development Core Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, 2009.
36. Wood SN. *Generalized additive models: an introduction*. Boca Raton, FL: R. Chapman and Hall/CRC Press, 2006.
37. Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. *nlme: linear and nonlinear mixed effects models*. Vienna, Austria: R Foundation for Statistical Computing, R package version 3.1-96, 2009.
38. Gilbert F. *Hoverflies. Naturalists' handbooks no. 5*. Slough, UK: Richmond Publishing Co., 1993.
39. Stubbs AE, Falk SJ. *British hoverflies*. Reading, UK: British Entomological & Natural History Society, 1996.
40. Simmons T, Cross PA, Adlam R, Moffatt C. The influence of insects on decomposition rate in buried and surface remains. *J Forensic Sci* 2010;55(4):889–92.
41. Simmons T, Adlam R, Moffatt C. Debugging decomposition data—comparative taphonomic studies and the influence of insects and carcass size on decomposition rate. *J Forensic Sci* 2010;55(1):8–13.
42. Goodbrod JR, Goff ML. Effects on larval population density on rates of development and interactions between two species of *Chrysomya* (Diptera: Calliphoridae) in laboratory culture. *J Med Entomol* 1990;27:338–43.
43. Greenberg B. Flies as forensic indicators. *J Med Entomol* 1991;28:565–77.
44. Haefner JN, Wallace JR, Merritt RW. Pig decomposition in lotic aquatic systems: the potential use of algal growth in establishing a post-mortem submersion interval (PMSI). *J Forensic Sci* 2004;49(2):330–6.
45. Forbes S, Stuart BH, Dent BB, Fenwick-Mulcahy S. Characteristics of adipocere formation in animal species. *J Forensic Sci* 2005;50(3):633–40.
46. Giertsen JC, Morild I. Seafaring bodies. *Am J Forensic Med Pathol* 1989;10(1):25–7.
47. Bachmann J, Simmons T. The influence of pre-burial insect access on the decomposition rate. *J Forensic Sci* 2010;55(4):893–900.

Additional information and reprint requests:

Tal Simmons, Ph.D.

School of Forensic and Investigative Sciences

University of Central Lancashire

Maudland Building

Preston, PR1 2HE

U.K.

E-mail: tlisimmons@uclan.ac.uk