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Case closed – Wrappings and encasement delays and reduces fly presence on body parts

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ABSTRACT

Homicide, particularly where a body has been concealed, is uniquely challenging for investigators to estimate the time of occurrence due to the methods employed by perpetrators to hide the body or its constituent parts from detection. The regularity of necrophagous insect lifecycles to determine minimum post-mortem interval (minPMI) is widely employed but remains an unreliable technique if used without a clear understanding of the factors that affect insect access and oviposition behaviour to concealed remains.

The purpose of this study was to investigate the effect of wrapping body parts on fly colonisation and implications for minPMI calculations. Field studies were carried out using four treatments of pork (as surrogate body parts), in five replicates, one unwrapped, the other three wrapped in either a black plastic sack, a small-zipped wash bag (to simulate a suitcase), or a plastic sack further placed in a wash bag. Over a 48-h period all the methods of wrapping significantly disrupted the host-finding process of blowflies to dismembered carcasses, with a delay of initial contact and oviposition of 30+ h (dependant on wrapping) and even more in wet conditions (48+ h). Egg numbers were also reduced by as much as 99.1% on wrapped samples compared to unwrapped. These new findings highlight the importance of applying adjustments to minPMI calculations when encountering wrapped remains. Advances in the accuracy of minPMI calculations will prevent the waste of valuable police time and resources and better focus the search for witnesses and suspects in homicide investigations.

1. Introduction

Once human life ceases, a body begins to decay. A number of intrinsically linked factors govern the timescale for this, including its resting place, the prevailing climate, and the type and extent of clothing [1–3]. Once a body has been found, especially a suspected homicide, a reliable estimate of the time of death becomes paramount in the search for potential witnesses and suspects. A century and a half's research [4,5] has still to find a single method which accurately determines the elapsed time between death and body discovery, the post-mortem interval (PMI). Historically pathologists favoured hypostatic body discolouration or the progression of rigor mortis to help define the PMI window, however, these have been shown to be extremely variable in both their onset and progression [6–8]. More recently, post-mortem biochemistry and genetics have begun to provide compelling evidence of their future potential for forensic death investigation [9,10], however, these methods still need further development if their usage is to become the standard approach.

The current, most relied-upon method for PMI determination is the body's rate of post-mortem cooling, but this process is also subject to significant variability caused by differing body dimensions, ambient temperature, the duration of the terminal episode, as well as the effects of environmental conditions [8,11,12]. However, after 36–48 h, a body has reached the ambient temperature of its existing surroundings and there is very little scope for any dependability [13]. Post-mortem interval estimations that involve longer timescales tend to lean on forensic entomology, where knowledge of insect behaviour and development may provide a PMI estimate months or even years after a death [14]. Necrophagous insects such as blowflies inform PMI estimates due to the predictability of their lifecycle in association with a corpse [15]. Blowfly species travel up to 20 km per day to utilise carrion as egg-laying sites, and are often attracted to a dead body within thirty minutes of death [16].

Once a gravid female fly has accepted the corpse as a suitable site, eggs, or sometimes first instar larvae, are laid in batches of up to

Abbreviations: PMI, Post-mortem interval; minPMI, Minimum post-mortem interval; GLM, Generalised linear model

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300, singly or in clusters, and positioned ideally in moist, protein-rich places where neonate larvae can thrive. Commonly this will be in natural orifices or wounds, or at the body/ground surface interface [17,18]. Hatching times of fly eggs vary according to temperature, but for *Calliphora vicina,* (commonly known as a blue bottle), a typical member of this genus, the first larvae have been shown to emerge around 24 h after oviposition at 15 °C [15]. Subsequent larval development is characterised by the rapid assimilation of the soft tissues of the corpse, with larvae feeding in aggregation [16,18,19]. Post-feeding third instar larvae usually disperse from the remains of a body to seek a suitable haven to pupariate [1,14,20,21]. It is this largely predictable process that allows for the calculation of a minimum PMI (minPMI) based on the state of the oldest larvae found on a body, which indicates the age of the larva at a given temperature [15]. Forensic entomologists always give minPMI estimates rather than attempting to predict the moment of death itself (actual PMI), as the insect-based calculations on which they rely can be influenced by a multitude of factors that directly affect an insects' detection of its host, and its oviposition and subsequent development on it. Most importantly the habitat or situation of a body can affect the extent to which it attracts insects. Remains hidden in water, buried, or otherwise concealed in wrappings such as plastic refuse sacks or zipped suitcases can restrict the release of volatile compounds that attract insects to oviposition sites [22,23]. Extremes of temperature, both ambient and internal to the decomposing corpse, can also bring about potentially fatal stress responses in insects which can modify insect behaviour through metabolic change [1,24–26]. The presence of predators may also slow the establishment of a blowfly population [27,28] whilst a predilection to either a sunny or shady habitat can also be attractive or deterrent influences for insects searching for places to breed and feed [29–31]. Carrion size also matters for insects, as the smaller, faster decaying carrion do not attract the same insect species that typically colonise larger carcasses [32–34].

The size of any remains is particularly important when criminal events have caused death, as homicide encourages rapid clandestine concealment of victims. A murderer with a dead body either disposes of it where it is killed or moves it to an alternate site to hide or dissipate the remains. Opportunities for places of concealment increase significantly the smaller and more compact the remains are [35] and this has been shown to encourage defensive dismemberment [36], the mutilation of a body to aid disposal or prevent identification [37–39]. Criminal dismemberment worldwide is relatively uncommon; Adams et al. [40] found that in New York from 2000 to 2016 (excluding the victims of 9/11), there was an overall annual homicide rate of 527 individuals, of which, 2.5 cases were caused by dismemberment (1:224). An earlier study from Germany (1959–1987) reported a lower frequency of 1:500 [41] whilst in Korea, a study from 1995 to 2011 saw 65 cases of mutilation out of 1200 recorded (1:18.5) [42]. In the UK, Black et al. [35] reported a total of 85 cases over a period of 32 years (1985–2017) with an average of 2.66 dismemberment cases per year. Although these numbers are relatively small, the uniquely dehumanising nature of dismemberment [35] still warrants extensive investigative time and effort. Although previous studies have found it difficult to attribute any consistent traits with homicidal mutilators, some research in Finland found an association with murderers who knew their victims before the crime, especially if they were partners or family members [39].

The structural composition of a corpse may challenge the accuracy of minPMI estimations. Rivers & Dahlem [16] note that fur or feather coverings on animals can inhibit insect oviposition and feeding, as they can prevent direct access to the moist internal structures (where a body is face down and natural orifices are obscured for example). In a human corpse, these natural obstructions are often replaced by clothing and where homicide is concerned; any additional wrappings that may have been added to aid concealment. This may significantly affect the starting point for any minPMI estimation based on insect behaviour and breeding, because previous oviposition delays of up to 3 days have been observed when access to a corpse has been blocked by wrappings, burial or other concealment methods [23,43–45].

Despite the indications that wrappings may disrupt accepted insect lifecycle timescales, little experimentation has been undertaken in field conditions, or with remains suitably sized to simulate dismembered body parts or those concealed in easily acquired wrappings, and this represents a clear knowledge gap in this field. This study therefore addresses this shortfall by investigating the attraction time and oviposition behaviour of blowflies to simulated bodies or body parts, either unwrapped, or wrapped in a plastic refuse sack, in a zipped suitcase-like container or a refuse sack inside a zipped container. Insect egg-laying in the first two days after death was examined under field conditions to evaluate the possibility of any delay in oviposition caused by these conditions which could impact minPMI calculations.

2. Materials and methods

2.1. Cadaver proxy and rationale

The dismemberment of a human body is most easily accomplished by dividing the body into six parts: with the torso being separated from the head and four limbs. The limbs can be further sub-divided at the elbow and knee joints [35]. Given that limbs are the least cumbersome to transport and conceal in easily accessed domestic luggage and also that pig carcasses have been found to be reliable experimental substitutes (models) for human flesh [46–48], four similarly sized shoulder (hock sections) of the domestic pig (*Sus scrofa domestica* Linnaeus); mean size 0.82 ± 0.018 kg, 95% C.I. (source: Preston Family Butchers, Canterbury), were used as surrogates for human limbs for each of the five replicates in this study. Care was taken that all samples were kept shielded from potential insect activity before experimental use. They were also kept chilled from the point of euthanasia until 30 min before the trial began when they were brought up to ambient temperature in a warm room prior to use.

2.2. Research site

All field studies were carried out in a rural, detached half acre of land in East Kent, UK. The experimental sites were positioned in four areas with similar, shaded aspects, at least 40 m apart and surrounded by garden annuals, shrubs, and bushes. The site was bounded by a mix of domestic dwellings and farmland.

2.3. Trial period

Each of the five replicates took place over a 48-h period between 16th July and 15th August 2017 with each repetition commencing at 10:00 on day 1 and terminating at 10:00 on day 3. Continuous observations took place from the start of the trial until the first insect contact with each sample was observed. Following insect contact, the samples were checked at subsequent 2 hourly intervals. If first contact had not been observed by 16:00 on day 1, the observations were extended to dusk and then resumed on day 2 at 06:00. This is in line with the findings of Baldridge et al. (2006) [49] who noted that fly interest in baits largely ceased outside of these hours. Although there is some blowfly nocturnal behaviour research which indicates that overnight oviposition activity may occur [50] the present consensus is that this is limited [51]. In only one case in our study was oviposition noted which could have occurred overnight, although it is also possible that this oviposition which was newly

found at the end of the trial on the morning of day 3 may have taken place shortly before the final observations were taken.

2.4. Cage exclosures

The experimental and control treatments were housed in four, black, wire, double door, pet cage exclosures: H61cm x W76cm x D54cm (Argos; J. Sainsbury plc) (Fig S1). The internal black plastic tray bases were upturned to prevent rainwater retention. The outside of each cage was further encased in 13 mm mesh (Galvanised Cage and Aviary Mesh, Gardman Ltd.) to allow for insect access whilst excluding unwanted scavengers such as birds and mammals that might disrupt the experiment. A medium meat roasting bag (J. Sainsbury plc) was placed in-between the mesh and the top left face of the cage to act as protection for a data logger OM-EL-USB-2-LCD©, (Omega Engineering Inc.) which was secured by cable ties underneath this bag on the top inside surface of the cage. Each cage was placed directly onto the soil in one of the four positions (1–4).

2.5. Environmental observations

Hourly temperature and humidity readings were automatically collected using the 'in-cage' data loggers and these readings plus wind-speed, rainfall and barometric pressure were also taken at the same times by a Wireless Pro weather station WMR89/89 A© (Oregon Scientific Global Distribution Ltd.), centrally positioned on the site.

2.6. Treatments

Three experimental treatments and a control were used reflecting different states of wrapping or enclosure of the pig meat in this study:

2.6.1. Control

One piece of meat (treatment A) (Fig. S2), representing human remains disposed of in an unclothed state and free of any coverings, was left unwrapped and placed into a plastic standard tray propagator lid (internal size 20 cm × 31.5 cm×7.5 cm, Desch, Plantpak Ltd), filled to 6.5 cm with building sand (grain size 125–250 μm, Wickes, UK).

2.6.2. Garden sack

Plastic sacks are a popular choice for wrapping relatively heavy items which may be wet, including body parts covered in blood [52]. For this second treatment B (Fig. S3), a pork piece was placed at the bottom of a heavy duty, tie handle, black plastic garden sack (J. Sainsbury plc). Despite its heavy-duty description, the plastic material appeared to be thinner in some areas when held up to the light (although there were no holes). An inspection of several popular brands of sack prior to the trial indicated that this was a normal manufacturing feature of plastic waste sacks. The tie handles were not used for this study, rather the sack was trimmed to 30 cm long for experimental ease. The mouth of the sack was gathered, twisted 5 times, and secured as tightly as possible with a 30 cm long black cable tie (P&M Brills Hardware, Birchington). Excess cable tie was trimmed back but still ensured secure closure of the sack. The sack was placed on a sand tray before being sited in a cage.

2.6.3. Wash bag

In order to simulate a small body or dismembered body part hidden inside a suitcase, a third piece of pork, treatment C (Fig. S4), was placed inside a black wash bag (Boots Little Black Bag Mens, L 22.5 cm x W14 cm x D 8.5 cm x H 13.5 cm, The Boots Company PLC). The 100% nylon outer and 100% polyester inner lining closely matches materials typically found in suitcase construction. The bag had an external, zip-close side pocket. The zip for the side pocket comprised coiled plastic teeth directly moulded onto a carrier tape with a black metal slider; there was no access to the main compartment from the smaller one. A similar configuration of zip (except the slider was silver metal) was used to close the main compartment of the bag. Care was taken to ensure that neither the meat, nor any blood products from it, came into contact with any area of the bag whilst setting-up the experiment, as a previous study has indicated that damp or blood contaminated zips may influence blowfly oviposition choice [23]. The wash bag was placed on its side (to mimic a heavy suitcase enclosing a body/part(s)), in a filled sand tray and positioned following the methodology previously outlined.

2.6.4. Garden sack plus wash bag

In order to give extra protection from prying eyes or the odour of decomposition, a murderer may choose to enclose body parts in several layers or types of wrapping. This was taken into consideration in treatment D of this study (Fig. S5), where a pork piece was wrapped in the exact manner of the plastic garden sack set-up (treatment B) and then further placed inside a wash bag (as treatment C), on sand and placed in the garden.

2.7. Repetitions

Four further repetitions of the two-day experimental period were carried out (5 overall). Each repetition saw conditions (A-D) placed in subsequent cage positions (1–4) around the garden according to a randomised Latin square design which ensured all treatments were tested in all positions.

2.8. Experimental design oviposition study

2.8.1. Time to first contact

All treatments (A-D) were placed into their experimental sites (1–4) and photographed. Recordings were made of the time of first blowfly appearance and subsequent contact with the external wrappings/unwrapped control. After first contact had been established, bi-hourly recordings were taken at all sites for the duration of the study (10.00–16.00) each day. The position of any eggs/egg clusters was recorded using photography. Wherever possible, the first blowflies to make contact were caught using a small hand net. Thereafter, a random subset of visiting flies from each condition across the 5 trials were sampled. Appropriate keys were used for identification [53,54].

2.9. Egg and larvae collection

2.9.1. Unwrapped sample-Control (A)

At the end of the two-day experimental period, the control pork was removed from its sand tray and any eggs or larvae seen adhering to the outside surface were removed using a damp paintbrush and counted. The entire surface of the meat was then carefully washed with water from a wash-bottle onto the surface of a black muslin cloth (The Range, UK) stretched over a plastic kitchen washing-up bowl and held in place with a large elastic band. This process was employed to remove all other eggs and larvae (a 10-minute period was allowed between each wash phase to enable larvae, stimulated by the water, to emerge onto the surface from within the inner crevices and folds of the meat). Once on the cloth, this apparatus served as a filter for the water/larvae/egg mix from the meat. Following filtration, the resulting eggs and larvae were spread evenly across a measured area of the cloth (the spread of the eggs and larvae depended on their number as a uniform thickness was sought to ensure consistency of sampling), and a 1 cm^2 sample of the insect progeny were carefully removed using a spatula. These were placed into a Petri dish and counted under a microscope. This process was

then repeated by another researcher taking a separate 1 cm^2 square sample from the same cloth; the eggs and larvae were recorded separately. The results were then averaged, and samples were taken for species identification.

2.9.2. Eggs/larvae from sand trays (A-D)

After the samples had been removed from the sand trays, any egg clusters, and larvae on the surface of the sand were removed using a spatula. The trays were then flooded with water to the top of the tray to enable any hidden eggs/larvae to float. These were collected using a large spoon. The sand was then gently agitated to dislodge deeper-positioned progeny. The remaining water was filtered away through muslin, as previously described, leaving any eggs and larvae to be counted. A sample of the collected eggs/larvae were taken for identification.

2.9.3. Outside of wrapped samples (B-D)

As the wrapped samples of meat had significantly fewer eggs and larvae present on the outside of their wrappings, it was generally possible to remove the progeny using the damp paintbrush method outlined previously, however the occasional difficult egg cluster did need washing free as before. Samples were taken for species identification.

2.9.4. Eggs/larvae inside wrappings (B-D)

To preserve eggs and larvae which had been laid inside the wrapped samples, the garden sacks and wash bags were carefully cut open with a scalpel in places where oviposition was least expected therefore avoiding the areas of closure around the cable ties and zips. Once exposed, the positions of the eggs/larvae were recorded and photographed and then counted after retrieval by one of the methods previously mentioned. A representative number of progeny were taken for identification as before.

2.9.5. Eggs/larvae on surface of samples B-D

Where eggs or larvae were present on the meat inside the wrappings, they were collected using the paintbrush method and counted singly.

2.9.6. Rearing to adulthood

Fifty eggs and fifty larvae were taken for each treatment, from each of the black muslin cloth, any wrapping, and the sand, and reared to identify the species of insect present (in some instances, larvae, eggs, or both were completely absent, so the overall sampling numbers appear reduced).

These samples were relocated onto pieces of pork hock (approximately 4 cm^2 from the same butcher). Using a moist paintbrush, the larvae or eggs were placed in individually labelled plastic containers with holes punctured in the top for ventilation. These containers were left in a shaded position in the garden. The meat was sprayed daily with water to prevent it drying out. Once wandering activity of third instar larvae began, course sawdust was added to one end of the box to facilitate pupariation. After eclosion, all insects were removed for counting and identification using appropriate keys [53,54].

2.10. Statistical analysis

The effect of the time delay to first blowfly contact on the number of eggs and larvae at 48 h was investigated using a linear regression with log transformations for both time and number, to produce an approximately linear relationship.

The effect of the wrapping method was estimated using a generalised linear model (GLM) with negative binomial errors and a log link [55]. Tukey-corrected multiple comparisons between wrapping methods were carried out using the R 'multcomp' package, based on the GLM model [56]. Time to oviposition effects was estimated using a censored regression model [57] with multiple comparisons carried out by a Holm corrected Tukey test [58] Statistical significance was taken as $p < 0.05$ throughout.

3. Results

Throughout the period of the field trials the ambient temperature ranged diurnally between 9.5 °C and 37.5 °C (mean = 22.4 °C). The weather was generally sunny and hot, however, there was considerable rainfall for extended periods of trial 2. Wind was generally absent to light except for trial 3 where long periods of gusting wind were recorded.

3.1. Insect attraction to meat

Of the blowflies caught and identified after first contact, *Lucilia sericata* (Meigen) (in the genus commonly known as green bottles) were the first to appear on all experimental samples. *Calliphora vicina* Robineau-Desvoidy and *Calliphora vomitoria* (Linnaeus) (both known as blue bottles) were also in evidence throughout the trials. Multi-comparison analysis shows differences in attraction times between the unwrapped samples and each of the other samples ($p < 0.0001$) (Fig. 1).

Blowflies (as identified above) were attracted to the unwrapped meat (A) soon after commencing the experiments with a mean contact time of 8 min 31 s (0.14 h) across the 5 replicates and the fastest contact recorded after 1 min 58 s (0.03 h). The garden sack treatment (B) showed the next fastest mean attraction time of 7.01 h. The wash bag (C) showed first contact at a mean of 20.99 h. The longest time before first contact was observed for the garden sack with wash bag treatment (D) at a mean of 25.68 h. The only day 1 contact for this double-wrapped sample occurred in trial 4 after 3.17 h.

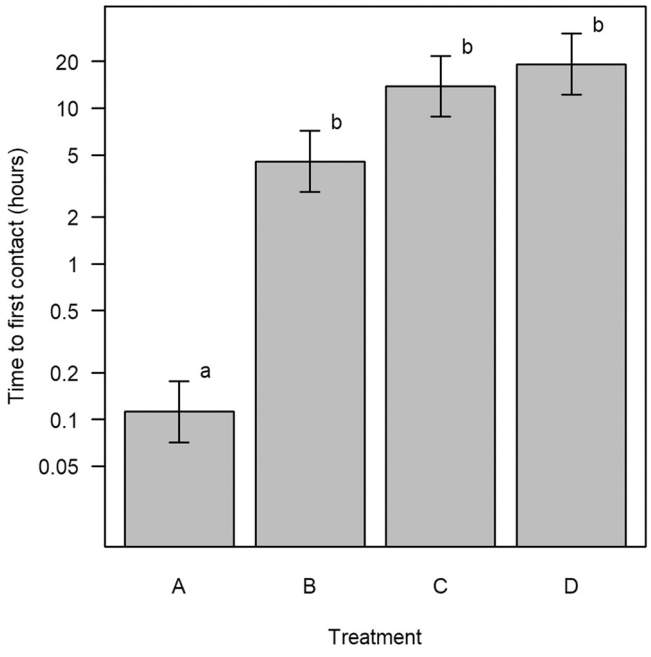

Fig. 1. Multi-comparison analysis of deviance for mean time (n = 5) of first blowfly contact to samples of pork according to wrapping treatment; Unwrapped (A), Garden sack (B), Wash bag (C) Garden sack and wash bag (D). This General Linear Model (GLM) analysis with binomial errors and a log link uses compact letter display codes to indicate which treatments differ from each other. Groups which do not share a letter are significantly different ($p < 0.05$). Error bars indicate ± one standard error around the mean. Log-scale standard errors predicted from GLM models.

Although there was some inter-treatment variability of arrival times across the replicates, the greatest inconsistency occurred in the rainy trial 2 where treatments typically saw extended times to first contact.

Although data was not systematically collected for this aspect, observational notes and photographic records indicate adult blowflies were generally in greatest abundance (25 +) on the unwrapped samples in the morning / early afternoons of day 1 when the meat was freshest. As the trial progressed, and the meat dried out; the increase in blowfly numbers levelled out (approximately 12–18 when any observation was made) and did not regain their peak day 1 numbers. This differed in the wet trial 2, where after initial interest in the first 4 h, blowfly numbers never rose above 9 visiting adults at a time, although adults were never completely absent on the uncovered meat even during heavy rain.

The common wasp, *Vespula vulgaris (L.),* was also a regular visitor in small numbers (1–10) (eight was common), chewing pieces from the unwrapped meat and moving the newly deposited blowfly eggs around, although no evidence was seen of any permanent egg removal or predation.

Interest in the garden sack was steady once contact had been established but with no more than 9 blowfly adults at any time. Fly attention for the wash bag was generally sporadic with a maximum of 6 insects present in any trial, although in trial 4 fly activity was continuous from first contact whilst still maintaining similar numbers.

The garden sack and wash bag combination had irregular blowfly visits throughout.

None of the wrapped samples received blowfly visitation in the wet periods of trial 2.

Although first contact for the garden sack had preceded the rainfall, no flies were attracted to its wet surface for the remainder of that trial and first contact for the wash bag and garden sack and wash bag only occurred on the morning of day 3.

A significant negative linear relationship was shown between the time taken for first blowfly contact to occur and the numbers of eggs and larvae present after 48 h ($p < 0.0001$, $R^2 = 0.66$) (Fig. 2).

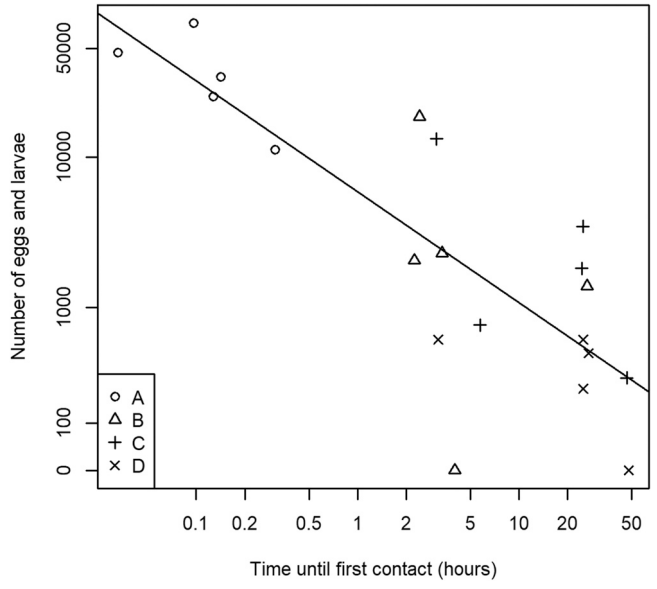

Fig. 2. Scatter plot showing negative linear relationship between time taken for first blowfly contact to occur and number of eggs and larvae present after 48 h on samples of pork: Unwrapped (A), or enclosed in: Garden sack (B), Wash bag (C) Garden sack and wash bag (D).

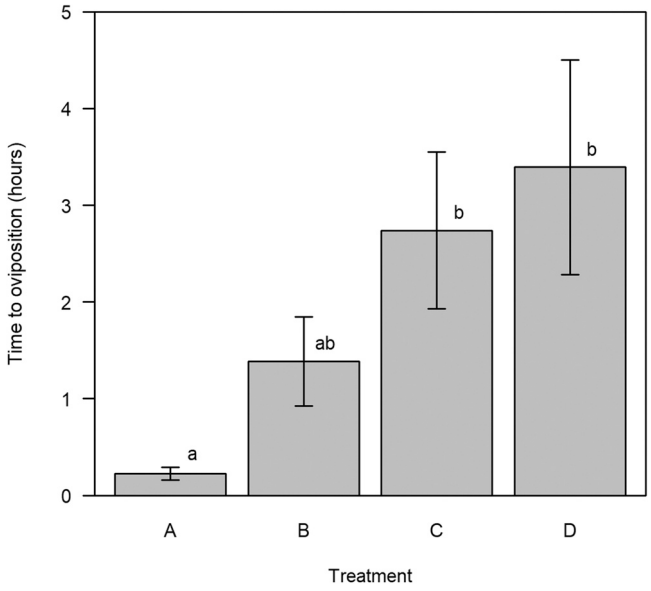

Fig. 3. Multiple comparison analysis of deviance for mean time (n = 5) of blowfly oviposition in samples of pork according to wrapping treatment; Unwrapped (A), Garden sack (B), Wash bag (C) Garden sack and wash bag (D). This censored regression model allows for two of the samples not having reached the oviposition stage by the end of the study. Compact letter display codes show which treatments differ from each other and were obtained using a Holm corrected Tukey test.

3.2. Oviposition times from start of trials

Time to first observed oviposition was lower in the unwrapped control (A) than for any other treatment ($\chi 2(3, N = 5) = 19.75$, $p < 0.0010$). The range of times to oviposition for this treatment varied between 0.18 and 4.00 h (overall mean of 2.23 h, 95% CI range 0.85–3.62 h). A Tukey HSD multiple comparison analysis indicated that the mean differences in progeny numbers between the samples was either; statistically significant $p = 0.016$ (mean of D with A), $p = 0.018$ (C with A) or not statistically significant (B with A, B with C, B with D, and C with D).

First oviposition times for the garden sack (B) varied between 4.00 and 28.00 h (overall mean 13.84 h, 95%CI range 4.18 – 23.50 h). For the wash bag (C), the minimum to maximum range was 3.17 – 47.92 h, with mean 27.4 h and 95%CI range 10.4 – 44.38 h. For the wash bag/garden sack combination (D), the minimum to maximum range was 5.75 – 48.00 h, with mean 33.93 h and 95%CI range 10.62 – 57.24 h (Fig. 3.).

3.3. Larval position

For the unwrapped meat, most eggs were laid in natural crevices and under flaps of skin or at the meat/sand intersection. These, cooler, shaded, and moist areas were favoured in almost all instances for egg clusters and for the location of eventual larvae, although solitary eggs were observed on occasion on the cut meat faces. Eggs on the garden sack were clustered together between its plastic folds, particularly near the cable-tied entrance. Larvae appeared to have no positional preference, wandering randomly across the surface of the sack and crawling on the sand. Some were seen making spiralling body movements whilst their mouth hooks were attached to the sack in an apparent attempt to reach the meat. For the wash bag and garden sack/wash bag combination; most insect attention was focused around the top and main pocket zip areas particularly at the end where the slider was situated. Eggs were observed both singly and in clusters both between the zip teeth and hanging down in the form of 'stalactites' [23] from the inside of the main compartment zip. This was a result of oviposition from the outside through the

spaces between the zip teeth. Eggs were also laid along the edges of the external piping at the bottom of the wash bags. In all cases there was a small gap where the top (smaller) compartment slider did not close completely against the bag exterior, and blowflies were seen disappearing into the top compartment via this opening.

Eggs were located inside the smaller compartment of the lone wash bag but rarely so for the wash bag and garden sack combination.

Eggs were found on the surface of the meat inside the wash bag (C) in trial 4; although these may have fallen from the inside of the zip, and larvae were seen crawling on the meat in trials 3, 4 and 5. Larvae were also occasionally found crawling inside the main compartment on the surface of the garden sack in the wash bag combination, although no eggs or larvae were found inside the garden sack at any time. In trial 2 affected by rainfall, eggs were observed only on the sand surrounding the lone wash bag and no eggs or larvae were recorded on the garden sack and wash bag combination.

3.4. Progeny numbers after 48 h

There was a significant effect of treatment on the numbers of progeny found ($\chi^2(3, N = 5) = 22.7$, $p < 0.0010$) with most found on the unwrapped control (A). Mean numbers for (A) varied between 11,186 and 72,880 (overall mean of 37,735.6, 95% CI [10.167 – 64,857]). Multi-comparison analysis and post-hoc Tukey HSD testing indicated that the mean differences in progeny numbers between the unwrapped samples and the wrapped conditions gave mixed results, with statistical significance shown, $p < 0.0010$ (D with A) and $p = 0.044$ (C with A), a figure approaching significance $p = 0.082$ (B with A) and no statistical significance 0.991 (C with B).

Egg/larval numbers for the garden sack (B) varied between 0 (trial 2) and 18,265 for trial 4 (overall mean 4828, 95%CI range 0–13,529). The mean differences of progeny for this treatment across the 5 trials did not differ significantly when compared with the wash bag alone (C) ($p = 0.995$) but when (B) was compared with the wash bag coupled with the garden sack (D) there were significantly more progeny in B ($p = 0.016$).

Numbers of offspring for (C) varied between 291 and 13,148 (overall mean 3917, 95%CI range 0–10,021). Eggs and larval figures for (D) ranged between 0 (trial 2) and 586 (overall mean 374, 95%CI range 82–666). There were statistically, significantly more progeny in treatment C than D ($p = 0.033$) (Fig. 4).

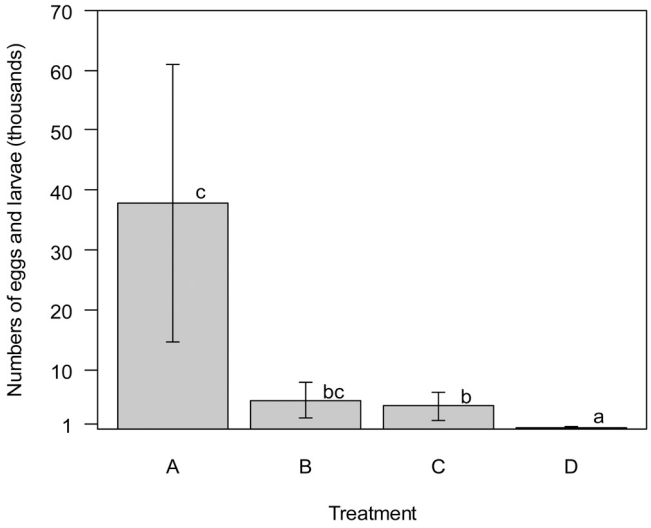

Fig. 4. Multi-comparison analysis of deviance for mean Calliphoridae egg and larval numbers after a maximum of 48 h blowfly exposure to samples of pork; Unwrapped (A), Garden sack (B), Washbag (C), Garden sack and wash bag (D). This General Linear Model analysis with binomial errors and a log link uses compact letter display codes to indicate which treatments differ from each other. Groups sharing a letter are not significantly different. Error bars indicate ± one standard error around the mean- Log-scale standard errors predicted from GLM models.

3.5. Adult numbers

Of the 1800 egg and larvae samples taken for further development (treatments B and D for trial 2 had no progeny), 1244 produced hatched flies. *Calliphora vicina* were in the majority with 785 of adults that emerged across the 5 trials, 399 of the overall sample were *Lucilia sericata* with just 60 *Calliphora vomitoria* present.

4. Discussion

4.1. Wrapping distorts minPMI

It was found that wrapping surrogate body parts significantly disrupted the host-finding process of blowflies to small or dismembered carcasses. The delay between mean first egg deposition in the controls (A = 2.23 h) and the most delaying treatment (D = 33.93 h) was almost 32 h and 48 + where heavy rain persisted. Egg numbers were shown to be reduced in wrapped treatments by up to 99.1% when compared to unwrapped controls. The delay between exposure to the environment and first oviposition, in addition to the reduced numbers of possible progeny available to colonise and support the decomposition of the enclosed remains, indicates that without a correction for wrapping, traditional minPMI calculation methods may be seriously underestimating the time since remains were dumped. This inaccuracy could have a direct impact on the ability to correctly determine reliable timescales for witness or suspect apprehension, falsely casting suspicion on others in the vicinity at the time and resulting in a costly waste of police time and resources and having a negative impact on homicide clear-up rates.

The extended time to first blowfly contact may question the traditionally accepted attraction and oviposition times between fly and corpse [59,60]. This supports the oviposition delay to carrion contained in a zipped suitcase observed by Bhadra et al. [23]. Meat contained in plastic garden sacks were the flies 'most favoured wrapped samples, despite forming an impenetrable barrier to both ovipositing adults and larvae. This opposes the findings of Scholl and Moffatt [61] where the sacks merely inhibited insect access rather than preventing it. The longer duration of that trial may have been a factor, and if this new study was lengthened, the spiralling actions of the larvae may ultimately have broken down the plastic barrier. This study also found the plastic cable tie closures impenetrable to insects, even though the folds around the mouth of the sack were a favoured oviposition site. This was in contrast to the results found by Scholl and Moffat [61], although twists were additionally applied to the neck of the sack before closure in this study which may have helped prevent larval ingress. Where plastic sacks containing a body or its dismembered parts are concerned, this study has shown that the traditional formulae used to estimate minPMI based on observed blowfly lifecycle development [62] are unsuitable and that an adjustment of 18 h should be considered to reflect these mean findings, especially if care has been taken in the sealing of the gathered mouth of the sack.

Volatile odours released by a corpse soon after death initiate a range of blowfly behavioural responses such as mating, feeding and oviposition [63,64]. With the progression of decomposition, this volatile profile changes in intensity and composition [65,66]. Female blowflies in particular are attuned to this variability during oviposition and identify odour signatures associated with the moist tissue conditions preferable for progeny success [63]. The relatively short period when these factors may prevail [67] helps explain the insects' speedy response times to the most favourable stimuli [30]. Mechanisms which restrict the release and subsequent discovery of

such odours can disrupt the ability of blowflies to capitalise on these conditions [1,3,68,69] and the results from the wrapping methods used in this study support these findings. The lack of larval penetration into the plastic sacks supports a level of physical and volatile obstruction to visiting insects [70], however, the varying thickness of the sack material may not have presented a completely impermeable barrier to decomposition odours [71,72]. This may explain the faster insect attraction times to these wrappings compared to the more robust washbags. Although the wash bags allowed gravid females to oviposit through the odour-permeable zip teeth, the extended initial attraction time for this wrapping overall suggests that the thicker material of the wash bag itself may have contained volatile odours for longer than the plastic sacks.

The extended delay in washbag attraction generally over that seen in the control, may have corresponded with the time taken for volatile compounds to leave the wrappings. The large number of eggs laid within the top pocket supports this, as flies searched for areas mirroring their preferred dark, odour-impregnated surfaces for oviposition [23,73], despite the improbability of their larvae ever reaching the meat. This supports previous research demonstrating the urgency of blowfly oviposition behaviour, where eggs have been laid around areas which allowed volatile escape, but insufficient space for physical access [21,74]. The eggs and larvae clustered around the washbag stitching in this study may also have been a result of oviposition related behavioural activity to reach the odour source.

4.2. Behavioural disruption of flies

The reduction in egg and larval numbers observed on the wrapped vs unwrapped samples was likely caused by the delay and reduced numbers of flies responding to the odours emitted by the wrapped samples. This coupled with the possibility of a further reduction in numbers due to using the less favourable oviposition sites separated from the meat itself [18], could confound PMI calculations derived from estimates of expected progeny numbers and larval sizes, based on conventional attraction times to exposed bodies [75]. Visual cues have also been shown to influence the choice of resource selection in the presence of odour for some insects, and the colour of the wrappings used in this study may have affected this behaviour. Previous research has given mixed results [76,77] but Benelli et al. [78] have shown that the black colour of the wrappings may have been an additional stimulus for the insects, as they may have been favoured over the pale unwrapped samples [79]. Future work with lighter coloured wrappings is needed but it is possible that fly attraction to the wrapped samples in this study, may have been positively influenced because of their darker, more preferential hue.

4.3. Influence of materials

Despite the ambient conditions being the same for all samples, the three wrapped treatments showed unique but consistent patterns of insect attraction, i.e., oviposition delay and decreased egg numbers; these effects diverging more from the unwrapped control treatment as the wrappings became increasingly robust. The wrapping construction may have contributed to this by changing the microclimate surrounding the enclosed carrion. It has been shown that prevention of a free flow of air around a carcass can block the normal gaseous exchange processes and impact on natural carrion breakdown activity. The potentially altered profile of the volatiles released may have changed the attractiveness of the samples, the gravid females' ability to find the meat and the numbers of eggs laid [80].

4.4. Effects of temperature

Blowfly growth and development is intrinsically linked to changes in temperature. Campobasso et al. [1] and Marchenko [81] highlight the unpredictability of egg and larval development in temperature extremes and Pedigo [82] cautions against using their lifecycle for minPMI estimations in these situations. This is particularly relevant for this study because, although the ambient temperature never went above 37.5 °C, a random check on the conditions inside the top pocket of one of the wash bags during a hot period, recorded a temperature of 51 °C. This is in excess of the critical thermal maximum for blowflies which, although variable between species, typically falls between 28 °C and 48 °C [18]. In addition, it may be that the metal zips of these bags were too hot to maintain physical contact with, in sustained sunshine, as flies were observed making aborted attempts to land. It is also possible that the effects of heat on the dark wrapping colour and construction material may have deterred or actually destroyed insects, thereby impacting on the final number of viable progeny. Although restricted by the scope and duration of this trial, it may be beneficial in future studies; to compare shaded and unshaded treatments, and to investigate variance in long-term larval survival associated with exposure to different types of heat absorptive wrapping materials.

4.5. Influence of rain

Blowfly abundance, flight activity and carcass colonisation can be inhibited or halted completely in moderate to heavy rain [3, 83–85]. This may suppress or disrupt olfactory signals from both decomposition volatiles and pheromonal cues [86,87] deposited by gravid females to encourage conspecific oviposition intended to promote cooperative larval feeding.

The findings of this study support the discrepancies possible in minPMI estimation in these conditions and suggest baseline calculations taken from first contact times should be increased by one to two days for wrapped samples, and as the unwrapped sample saw no new insect or egg laying activity after the last pre-rain arrival, an extension should also be included for unclothed bodies. Whilst the severity of the wet weather needs to be considered in other studies, this finding is in direct agreement with the delay observed by Mahat et al. [88] but demonstrates that further work needs to be done in this area, as it contrasts with the work of Reibe and Madea [89] who found no significant effect on the number of egg batches in wet conditions. After heavy rain, the eggs found on the unwrapped sample were either in natural holes and crevices in the meat or on the surrounding sand. As no other eggs were found on the sand in drier conditions, it is most likely that the rain dislodged them from the meat. Flight activity and egg positions were unaffected by light showers, which is borne-out by the resistance of egg masses to wash-off attempts during egg collection and supported by the need for soaking in larval debridement therapy culturing, to dissolve the glycoprotein adhesive layer [90] which binds blowfly eggs to each other in egg masses [91]. These findings have implications for the retrieval activities of forensic crime scene personnel, who should consider wider search patterns when looking to collect egg samples for analysis from homicide scenes after wet weather.

This study has shown that the nature of the materials which form cadaver wrapping is an important factor in the time taken for flies to find dismembered remains. As plastic sacks are favourite wrappings in homicide cases, extended studies should be undertaken using sacks of varying thicknesses, to investigate the timing and mechanism of any larval access to bodies wrapped in this way. Great care was taken in this study to prevent damage to the plastic sacks whilst positioning the samples, however it is likely that a murderer anxious to dispose of body parts may not be so particular. Any resulting holes in the plastic could change the observed blowfly

attraction and oviposition timings completely, as direct access to the flesh and an unrestricted and unchanged volatile profile may present conditions more akin to unwrapped samples thereby giving a timing of body deposition rather than a minPMI. Further work should therefore be undertaken with more realistically (hastily) wrapped carrion to improve the accuracy of any minPMI calculations extrapolated from plastic sack findings.

Although the wrapping of bodies has been previously considered as a confounding factor in minPMI estimation, there has been little focus on the actual effect on progeny numbers according to wrapping type. This study provides evidence that the presence of wrapping, and the make-up of those wrappings does cause delays in oviposition which reduces the average number of eggs laid in a 48-h period and affects subsequent larval development. This could prove confounding to police investigators if not factored into homicide or unexplained death calculations and in an age where police resources are stretched to their maximum, information which provides an accurate and speedy response to violent crime is paramount. Although investigating entomologists would routinely take account of any wrappings that could impact subsequent insect activity in their minPMI estimations, how precise that impact might be remains largely unclear. Given the findings of this study, there is undoubtedly a need for further research of the sort described here, to better determine these impacts for the future.

5. Conclusion

The ability to utilise flies as forensic indicators of time since death, hinges on securing the most accurate estimation of first oviposition between insect and the deceased. This research has shown that wrapping and most importantly, the choice of material and method employed to wrap a body or its dismembered parts, is crucial to the time taken for blowflies to find and complete oviposition on them. Although the samples in this study represented a body that had already cooled to ambient temperature, this may not be an uncommon state in an actual homicide when a murderer has taken the time to wrap a corpse in a suitable way for disposal or concealment. All wrapping explored in this study significantly lengthened the time taken for blowfly colonisation to occur. If these findings are not accounted for when bodies are found in a wrapped state, there is a danger that inferences applied from traditionally expected oviposition and progeny numbers on unwrapped bodies could seriously affect the accuracy of minPMI estimates and the investigative judgements resulting from them.

CRediT authorship contribution statement

Linda Brownlow: Investigation, conceptualization, Writing – Original Draft. **Stephen Young:** Formal Analysis. **Mandela Fernández-Grandon:** Supervision, Writing – Review and Editing. **Richard J. Hopkins:** Supervision, Conceptualization, Writing – Review and Editing.

Conflict of interest

No other conflicts of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.forsciint.2022.111542.

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