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Optimising crime scene temperature collection for forensic entomology casework

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Highlights of 'Optimising crime scene temperature collection for forensic entomology casework'

1. We illustrate the complexity of estimating insect exposure temperatures on a body.
2. Estimating the temperatures of bodies exposed to direct sunlight is problematic.
3. Loggers should be placed at crime scenes with caution; further research is needed.
4. A logger shelter seems to be advantageous only with direct exposure to sunlight.
5. Loggers should be placed on site in as similar a position as possible to the body.

Abstract

The value of minimum post-mortem interval (minPMI) estimations in suspicious death investigations from insect evidence using temperature modelling is indisputable. In order to investigate the reliability of the collected temperature data used for modelling minPMI, it is necessary to study the effects of data logger location on the accuracy and precision of measurements. Digital data logging devices are the most commonly used temperature measuring devices in forensic entomology, however, the relationship between ambient temperatures (measured by loggers) and body temperatures has been little studied. The placement of loggers in this study in three locations (two outdoors, one indoors) had measurable effects when compared with actual body temperature measurements (simulated with pig heads), some more significant than others depending on season, exposure to the environment and logger location. Overall, the study demonstrated the complexity of the question of optimal logger placement at a crime scene and the potential impact of inaccurate temperature data on minPMI estimations, showing the importance of further research in this area and development of a standard protocol. Initial recommendations are provided for data logger placement (within a Stevenson Screen where practical), situations to avoid (e.g. placement of logger in front of windows when measuring indoor temperatures), and a baseline for further research into producing standard guidelines for logger placement, to increase the accuracy of minPMI estimations and, thereby, the reliability of forensic entomology evidence in court.

Keywords: forensic science, minPMI estimation, Stevenson Screen, temperature data logger, United Kingdom, body temperature, logger placement

1. Introduction

The study of insects and other arthropods in a medico-legal context, also known as forensic entomology, is an essential tool in legal cases, especially in death enquiries [1]. Traditional forensic pathology methods for time of death estimation, using post-mortem changes such as rigor mortis, reach their limits within 48-72 hours after death [2]. At that point forensic entomology becomes particularly important as knowledge of insect biology, ecology and identification can provide information such as post-mortem body relocations, environmental conditions at death occurrence and, most importantly, estimation of a minimum post-mortem interval (minPMI), i.e. the minimum time elapsed since death occurred, equivalent to when the indicator insects first infested the body. Recognition of the importance that insect specimens found on or in proximity to human cadavers are considered physical evidence and should be processed as such has been reported in the literature repeatedly over the years [3, 4]. In forensic entomology, as in all branches of forensic science, a common frame of guidelines and standards for collection, packaging and transport, preservation and identification of insect evidence is essential to ensure good practice and applicability in legal cases [4, 5, 6]. It is important that these guidelines and standards are always amended to what is understood as 'best practice' to date.

The effects of seasonal temperature variations on decomposition of remains have been reported in

a study in 2004, which observed that higher temperatures and rain increased decomposition rates [7]. As insect evidence involves working with living organisms, these are, naturally, influenced by external factors that determine their growth and development. The main factor influencing insect development rates and adult behaviour is temperature, because they are poikilothermic, i.e. their internal body temperature fluctuates with varying ambient temperatures [8, 9]. The importance of temperature in entomological investigations has been reported repeatedly in literature relating to this subject area - Hall *et al.* [10] provide a list of relevant publications. Not only is knowledge of scene temperatures essential for accurate minPMI estimations, it is also important for estimations of the pre-appearance interval (PAI) in insect succession patterns [11, 12]. Developmental rates vary between lower and upper temperature thresholds specific to each species, and can cease completely if the temperature falls below or rises above these, respectively [13]. Temperature development rates may also vary within the same species among different geographic regions.

Blow flies (Diptera: Calliphoridae) are used predominantly in forensic entomology for minPMI estimations by use of larval lengths as 'biological clocks' [10, 14]. The insect species mainly studied for forensic entomological purposes in the UK are the most commonly occurring blow fly species: *Calliphora vicina* Robineau-Desvoidy, 1830, which can be active in all seasons, *Calliphora vomitora* (Linnaeus, 1758) and *Lucilia sericata* (Meigen, 1826), both of which can be found only from early spring to late autumn. The minimum temperature threshold for development of *C. vicina*, the most common blow fly in urban areas in the United Kingdom, has been estimated to be 1 °C by Donovan *et al.* [14] and, based on their data, a variation in temperature of just ±1 °C at average temperatures of 21 °C can significantly affect the duration of development by ±5 %. Thus, it is essential that the recording and analysis of temperatures affecting insect development provide accurate data for estimating development periods and hence minPMI [15].

For accurate estimations of minPMI it is therefore crucial to obtain measurements with the best possible confidence of the actual temperatures the insects developing on the body experienced at the scene [10]. However, this is complex because the relationship between ambient temperatures, measured by scene loggers, and body temperatures is poorly studied and there can be many confounding factors such as larval mass temperatures. The formation of larval masses inside cadaverous material can cause an increase in temperature, affecting both the body's decomposition rate and the larval development rate – this has been studied with increasing sophistication, but it is still difficult to account for on a case by case basis [10, 16, 17, 18, 19, 20, 21]. Suggestions for improvement in temperature estimation include optimal temperature data collection methods to increase accuracy of minPMI estimations as well as assessment of the reliability of data variability in different environmental and geographical conditions, seasonal differences, microhabitats, circumstances of death and decomposition rates [22, 23]. Other studies have looked at the relationship between temperatures recorded at scenes and those recorded at the nearest meteorological stations, with the objective of establishing a reliable relationship so that ambient scene temperatures can be estimated from meteorological station data [24, 25, 26]. However, the relationship between ambient and body temperatures has been little researched and that is the focus of the present study.

In the UK the current recommended practice for temperature data collection at outdoor crime scenes in forensic entomology is the use of temperature data logging devices protected by a Stevenson Screen (SS) [10]. A SS is a naturally vented shelter designed for protecting meteorological devices against environmental conditions, mainly direct sunlight, which could influence and alter measurements. The SS can be made of wood or plastic and is available in many different sizes. The SS often used in forensic entomology casework in the UK is smaller than those used in meteorological observations as it is specifically designed for sheltering small digital data loggers (e.g. ACS-5050 Datamate Weather Screen; Figure 1 – see unsheltered pig head).

Recent studies suggest that different factors, such as size of a SS [27] and sheltering/non-

sheltering of data loggers [28], can have an impact on logger performance. The former study showed that medium-sized wooden SS tended to provide approximately 0.5 °C higher daily maximum air temperatures than large-sized SS, while the latter study concluded that data logger results are inaccurate when measurements are made without shelter or under shelters that do not meet World Meteorological Organization criteria. Currently, there is a lack of studies dealing with optimal logger positioning at crime scenes. This is a potentially important factor that, if optimised, could improve current practices in entomological casework used by police forces and/or forensic entomologists attending crime scenes. Furthermore, assessing and increasing the accuracy of estimating body temperature retrospectively from measurements of ambient temperature is essential in strengthening forensic entomological estimates made from insect evidence.

The applicability of forensic entomology in legal and medical cases relies on an emphasis of good practice using a common framework of guidelines and standards [1, 4], with the improvement of data collection being an important factor in increasing inference strength and optimising results [29]. However, no standard practice protocol includes guidelines for where to place temperature collection devices at crime scenes. The objectives of this study were to investigate the accuracy of estimating body temperatures using the current practice in ambient temperature measurement at crime scenes (data logger sheltered in a SS) in comparison with unsheltered data loggers exposed to the environment in different locations at a scene. The results provide guidelines for optimised scene temperature collection with regards to measurement device location for practical applications by police forces and forensic entomologists, in casework and research. This optimisation will aim to increase the accuracy of minPMI estimations and therefore contribute to their validity and the ability of expert witnesses to defend the application of forensic entomological evidence in casework in court [30].

2. Material and Methods

2.1 Experimental design

The natural body orifices, including those of the head, are the sites of initial blow fly colonization [2], where significant larval masses can form. Therefore, heads of adult pigs (4.7-7.2 kg; source, Turner & George, London) were used as a model for the heads of human cadavers [31, 32]. Hourly temperature data, measured on the hour twenty-four times a day, was collected from in and around the heads using factory calibrated Tinytag Plus 2® data loggers. The heads were placed in the environment at 14:00-15:00 hours on day 1 and checked each day at 10:00, 12:00, 14:00 and 16:00 hours in the summer or at 12:00 in the winter. During each checking period the heads were carefully examined visually and the presence of adult activity or of fly eggs was recorded. The measurements were conducted over a period of eleven days (N=10). The first five replicates were carried out from 16.06.2015 until 04.09.2015, and were termed the 'summer experiment'. The second five replicates were carried out from 22.09.2015 until 27.11.2015, and were termed the 'winter experiment'.

The collection of temperature data from pig heads was performed under the three following different environmental conditions on a 0.88-2.90 m wide balcony, 26.74 m above ground level, of the southwest tower of the Natural History Museum in London, United Kingdom (Figure 1):

- Outdoors on the balcony in an area exposed to sunlight from approximately 11:00-17:00 hours daily (unsheltered).
- Outdoors on the balcony in an area never exposed to sunlight (partially sheltered).
- Indoors (sheltered) in a room a further 3.7 m above the balcony.

The room was unheated and had two east facing windows, one of which was left partly opened throughout the study. Each replicate was carried out with one pig head for each condition simultaneously. Illuminance (luminous flux - lux) was measured daily at 10:00 hours in each location

for the duration of both experimental sets using a data logging Heavy Duty Lightmeter (HD450) with the sensor aimed upwards vertically.

Tinytag Plus 2® data loggers (55 x 50 x 33 mm) were placed (Figure 1, Table 1) to determine possible effects of logger location on temperature measurement. For each environmental condition, five Tinytag Plus 2® data loggers were used for temperature collection, assigned to different locations in a controlled Latin square design between replicates: one in an area exposed to sunlight on the ground (herein after termed 'sun' logger), one in an area sheltered from direct sunlight on the ground (hereafter termed 'shade' logger), one in an ACS-5050 SS (a cylinder-shaped shelter constructed of eight white plastic plates, 130 mm tall x 198 mm diameter; hereafter termed 'SS' logger) 1.25 metres above ground (i.e. balcony floor), according to a minimum height recommendation by the World Meteorological Organisation (WMO) [33], and two Tinytag Plus 2® probe loggers per head, one penetrating fully into the left cheek muscle and one placed fully into the left nostril, for measuring body temperature (probes were 100-150 mm in length). For both outdoor conditions – unsheltered and partially sheltered – the three external loggers were shared (Table 1).

The decomposition process of the pig head indoors was monitored using a calibrated NEC Avio Infrared Thermography H2640 thermal imaging camera to record surface temperatures [32, 34] once daily over the duration of the experiment (Figure 3). Verification of the source of hot spots (adult flies or larval masses) was made by direct observation during imaging. The outdoor heads were not monitored in this manner because of limiting factors such as inclement weather and image distortion due to reflections in bright sunlight.

2.2 Statistical analysis

Statistical analysis was carried out using Tinytag Explorer 4.9 (Gemini Data Loggers UK, 2003–2014), Microsoft Excel v. 14.0 (Microsoft, 2010–2016) and Unistat 6.0 (UNISTAT Ltd., 1984–2015). The objective of data collection was to determine how well different records of ambient temperature (in sun, sheltered or in SS) reflect and could be used to estimate the temperature of a 'body' exposed in a variety of situations (sun, shade, indoors), without confounding factors, such as pre-exposure storage temperature of the pig heads, and larval mass effects. Therefore, the data of all twelve data loggers was adjusted by discarding the first twenty-four hours and the last 120 hours, to negate storage and larval mass effects, respectively (Figures 2, 3), that would alter results by introducing artefacts to body temperature. After this adjustment, 120 hourly data points remained for each data logger. As each logger had its own measurement error, revealed during calibration, each data point was adjusted with a logger specific correction factor to minimise device error influences on data analysis. Correction factors were determined by calculating the variation between measured temperature and actual temperature when in a controlled environment (incubator set at 20 °C; Figure 2-D) over a period of four days, following each replicate. Application of the correction factor comprised a simple addition or subtraction of the error recorded by each logger when compared to the true ambient (incubator) temperature measured by a factory calibrated data logger. Following data correction, normality testing was performed using the Kolmogorov-Smirnov test. Statistical significance was determined using Bonferonni corrected *t*-tests (paired, 2-tailed) with a limit of significance at $p<0.05$.

A review of a UK forensic entomology case (3.2) was undertaken to establish what level of temperature measurement error would have a practical 'biological/forensic significance' for the specific purposes of this study. For example, while it might be possible to show that a mean difference of just 0.05 °C between two sites with stable temperatures was statistically significant, such a small difference would be unlikely to have a forensic significance in estimating minPMI.

To assess how well different measures of ambient temperature matched our measures of body temperature, a comparison of the actual body temperatures with those of the sun, shade and SS loggers was performed using a refined index of agreement (d_r [35]). This index was developed for evaluation of climatic models (here our sun, shade and SS loggers) against reliable observations (here our body loggers), to indicate the magnitude of difference between model-predicted and

observed values. The d_r index is bounded by –1.0 and +1.0 and dimensionless, with the lower limit indicating poor data agreement and the upper limit indicating good data agreement [35]. According to Willmott *et al*. [36], the *d*r index can be applied to any set of pairwise model-predicted and observed values as it has comparable units and its values can also be compared across studies. Additionally, mean absolute error (MAE) was calculated to assess logger performance [37]. For data visualisation, a Principal Component Analysis (PCA), which uses a specified set of correlated observations (principal components), was conducted to investigate the closeness of relationship of internal loggers to external loggers according to their variance (Figure 4).

3. Results and Discussion

3.1 Illuminance (lux) monitoring

Table 2 shows the average (and range) daily measurements of lux for the unsheltered, partially sheltered and sheltered locations. These measurements correspond with expected values in locations with direct sunlight (unsheltered), shaded sunlight (partially sheltered) and filtered sunlight (sheltered – indoors) [38]. This monitoring was conducted to confirm quantitatively that the unsheltered location had a genuinely greater illuminance on the surface of the pig heads than the partially sheltered one, both of which experienced greater illuminance than the sheltered pig heads indoors. The measurement of a slightly higher average lux in the winter than in the summer in the unsheltered location (Table 2) was most likely because lux readings were taken at 10:00 hours, before the unsheltered site received direct sunlight, and because the summer period was generally cloudy whereas the winter period, although variable, included some very bright days with comparatively high illuminance.

3.2 Case study for determining forensic significance limits

This case study was prompted by our analysis of data from Donovan *et al* [14], which indicated that an under- or overestimation of developmental temperatures by just ±1 °C could affect the rate of insect development on a body by ±5 % (see Introduction) - this would result in similar magnitude errors in minPMI estimation that could mislead a criminal investigation. In order to investigate the forensic significance for *C. vicina* of a small difference (in °C) between actual developmental temperatures and estimated temperatures (used to estimate minPMI), a study was conducted on an anonymised murder case investigated by a UK police force.

The victim was last seen alive in late afternoon on the 16th of a month in spring and their body was found on the 24th of the same month in small woodland. A forensic entomologist was instructed to estimate a minPMI using blow fly specimen evidence collected at the scene as well as at the post-mortem. In the final report to the police force, the forensic entomologist estimated the minPMI, based on the size of larval *C. vicina* according to the model of accumulated degree hours (ADH) and retrospective linear regression analysis using the closest weather station temperature data [4], to be between 04:00 GMT on 17th and 07:00 GMT on 18th of the month in question. As it is known that blow flies are not usually active during night time hours [39, 40], it can be reasonably expected that the initial blow fly colonisation of the body was most likely during day light hours on 17th.

The forensic significance of a temperature difference, i.e. its impact on estimating a minPMI, is a hypothetical consideration and clearly depends on the fly species and the average ambient temperatures. At lower temperatures the impact of any specific temperature difference will be biologically and forensically more significant than at higher ambient temperatures. In this case study the daily average temperature was 11.2 °C (range 3.6-16.9 °C) and the main forensic

entomology evidence (i.e. oldest stages) were larvae of *Calliphora vicina*. It was calculated that a temperature difference of just ±0.5 °C (i.e. adding or subtracting 0.5 °C from the scene temperatures) produced a ±4.4-9.0 % difference in the estimated age of the larvae (i.e. 7.5-15.5 hours difference over 7.1 days), hence also in the estimated minPMI. Thus, if the larval age was reduced by 15.5 hours the estimated minPMI was altered to the period 19:30 GMT on the 17th to 22:30 GMT on the 18th (most likely first oviposition on the 18th), while if the age was increased by 15.5 hours the estimated minPMI was altered to the period 08:30 on 16th to 11:30 on 17th (most likely first oviposition on the 16th).

This case study showed the importance of accuracy in temperature measurements at the crime scene after body discovery, as well as reliable weather station data for retrospective temperature regression estimation of the scene temperatures prior to body discovery. It also supports the assumption that differences in measurement of actual temperatures of as little as ±0.5 °C can be of forensic significance, giving rise to incorrect intelligence to the initial stages of police investigations, which could lead to incorrect inclusion or exclusion of potential suspects. Therefore, for the purposes of this study, differences of ±0.5 °C were considered forensically significant.

3.3 Assessment of Tinytag Plus 2® data logger performance for estimation of body temperature

In our study we recorded fly egg deposition (*Calliphora vicina)* on 29/30 pig heads within 22 hours of exposure, sometimes within 1-3 hours (6/15). Adult flies often arrived on the head within 1-5 minutes (19/30). These data are justification for the removal of the last 120 hours of each study due to larval mass effects (Figures 2 and 3).

Table 3 shows mean temperature differences (MTD's) ±95 % confidence intervals (CI's) and *t*-tests (Bonferonni corrected) for assessment of statistical and forensic significance between the actual body temperature and the ambient temperature measured in different locations. Refined indices of agreement, d_r, are shown in Table 4, and the mean measurements ±95 % CI's and the MAE for each data logger for both summer and winter experiments are shown in Table 5. In general there was a good match between the statistical and forensic significances, the latter being relevant to casework (Table 3).

The results of this study show that if a body is lying outdoors in an unsheltered location (US) in a warm weather period (summer experiment), all three external data logger locations are statistically or forensically significantly different to at least one of the actual measures of body temperature (Table 3), i.e. more than 0.5 °C difference in temperature measurements, which could lead to erroneous minPMI estimations (see 3.2). However, the external dataloggers reflected the body temperature accurately for bodies in partially sheltered and sheltered situations, apart from the sun logger indoors, which gave statistically and forensically significantly higher temperature readings (Table 3).

In a cool weather period (winter experiment) none of the external data loggers gave statistically or forensically different readings to the body loggers, apart from the shade and SS loggers which were statistically, but not forensically, different to one of the body temperature readings in the partially sheltered body (Table 3). This suggests that placement of data loggers during winter periods is not as critical as in the summer period in order to collect ambient temperature data that would be representative of the actual temperature the insects feeding on the body were experiencing. Part of the reason for the good relationship of ambient data logger temperatures to those of the internal, body temperature, dataloggers in the winter is that the period of daylight, and potential solar heating of loggers and bodies, was much reduced in winter compared to summer.

The generally small but sometimes significant differences between the two body temperature loggers, related to their position in either the nostril or muscle, suggests that caution should be exercised when estimating body temperatures. Clearly a body does not have the exact same temperature throughout its entirety and, of course, the insects do not always stay at the exact same spot during their development.

A study conducted by da Cunha [28] on temperature measurement errors of data loggers under different environmental conditions found temperature measurement inaccuracies in loggers without shelters, or with shelters not adhering to the WMO criteria [33]. However, no study has been conducted to investigate optimal positioning of data loggers at crime scenes, and the applicability to casework of sheltering data loggers in a SS adhering to WMO guidelines [33]; therefore this was one objective of the present study. In addition to the results discussed above, this was assessed by calculating d_r-indices and MAE to determine accuracy and precision of temperature measurements, hence overall performance. The d_r-indices were calculated to investigate temperature measurement accuracy of the internal logger probes compared to the externally placed loggers (Table 4). The d_r-indices show that in unsheltered and partially sheltered locations outdoors in warm weather periods (summer), the performance of an external logger in a SS is slightly poorer in comparison to a logger in the sun or in the shade when measuring actual body temperatures. In general, all external loggers showed poorer performance in an unsheltered location in the summer, but with the poorest performance shown by the sun logger in the sheltered indoor location, which is most likely due to direct solar radiation onto the measuring device as the sun logger was directly exposed to sunlight through a glass window, as in a glass house. During cool weather periods (winter), all external loggers showed good performance in every location, which supports the argument of interference in accuracy by direct sun radiation in summer periods. As the d_r- index ranges from -1 to +1 and all calculated d_r-indices were in the positive range, generally it can be said that logger performance was overall moderate to good in every scenario for both seasonal periods. The MAE was calculated for each logger to assess the average magnitude of total error, i.e. its precision (Table 5). It can be observed that MAE is generally higher in a cool weather period (winter) than in a warm weather period (summer), probably due to a higher range in temperature variation in the winter as can be seen when comparing the mean ±CI between summer and winter loggers (Table 5). Also, MAE is higher in loggers exposed to the environment, such as both external loggers in the sun and the unsheltered pig head internal probe loggers, for both summer and winter periods, which demonstrates that environmental factors, especially solar radiation, affect ambient temperature measurements.

The closeness of relationship between the internal and external loggers was examined using PCA (Figure 4). For the data collected in this study, two principal components were sufficient to describe >95% of variance. This analysis demonstrated that the outdoor logger placements in the unsheltered and partially sheltered locations form a distinctly different group to the indoor logger placements in the sheltered locations, for both warm and cold weather periods (summer and winter seasons) and for both datasets combined. Also it can be observed that, as noted previously, the actual body temperature measurements of the same pig heads using two different logger locations (PM and PN) are not identical, and sometimes show high variance between them, as in the case of both sheltered internal data loggers in the summer and both partially sheltered internal data loggers in the winter. The variance between the two internal loggers is not as distinct in the combined plot, however the PM and PN loggers in the sheltered location still show noticable variance (Figure 4). This clearly suggests that caution needs to be taken when discussing “actual body temperature” or “actual temperature the insects feeding on the body were experiencing”. Furthermore, these data also confirm that measurements of the external logger in the sun in the sheltered location are highly variable compared to all other loggers in the sheltered location for both seasons (Figure 4). As discussed above, it is clear that exposure to solar radiation through a

glass window can have a significant effect on ambient temperature measurements using data loggers as MAE values were high, and d_r-index values were low, when compared to the other loggers, indicating low performance accuracy of modelling the actual body temperature and a high total error. Another notable result is that of the internal PN logger in the sheltered location in the summer, which showed high variance to all other loggers in this location and season. As the effect of larval masses, which produce heat, was removed by data cutting (2.2), the mechanisms behind this require further study.

A study of the impact of SS size on air temperature measurement found that medium-sized SS tended to overestimate daily temperatures in comparisons to large-sized SS [27]. The SS frequently used in forensic entomology casework in the UK, and used by us here, is smaller than those described by Buisan *et al.* [27]. Our study is the first to investigate its applicability to forensic casework. The SS used was plastic and all white. It has been shown that plastic screens are, in temperate climates like the UK, a climatologically consistent alternative to wooden SS [41]. A comparison of wooden and plastic SS concluded that plastic SS, either with all white louvres (as here) or with white outer and black inner louvres, gave minimum temperatures that were not significantly different to those recorded using a wooden SS [Results of an inter-comparison of wooden and plastic thermometer screens by DB Hatton, Met Office Beaufort Park, presented at the World Meteorological Organization (WMO) Technical Conference on Meteorological and Environmental Instruments and Methods of Observation (TECO-2002). ©Crown copyright]. The maximum temperatures in the all-white plastic screen were nearer to those of the wooden SS than were those of the white/black plastic SS. Further research is necessary to investigate if the size and/or type of the specific SS used here is optimal for forensic entomology applications. However, it is clear that the SS logger provided a good estimate of body temperatures, except for those of a body lying in direct sunlight (Tables 3, 4, 5 and Figure 4). Although, other than the sun logger indoors in summer, the logger in a SS did not perform better in estimating ”actual” body temperatures of unsheltered or sheltered bodies than did the other external loggers (sun and shade) (Table 3), where possible an SS should be used as its use is in line with meteorological standards [33] and there is no disadvantage, save in discrete placement at a scene that cannot be maintained or protected from public interference. At unprotected scenes an SS could attract unwanted attention leading to the theft of loggers. However, in such circumstances the more discrete placement of data loggers not in a SS can still provide sufficiently accurate data (Table 3), especially in cooler months. Cable ties with seals securing an SS or logger to a fixed point at the scene could be used to prove that no interference had taken place; additionally a logger could be sealed inside a SS with such seals.

Further research over different months is suggested as the available seasonal data was limited to June-November and such studies should be repeated for at least three different years in the same seasonal periods. Also, this research was limited to temperate UK conditions and should, therefore, be repeated for other climate zones and different natural sites, such as open grassland and woodland, for developing, if necessary, country- and site-specific casework recommendations. Further research should also be conducted on the temperature differences experienced by insects feeding at different locations on the same body, in part due to larval mass effects. However, in this study we deliberately excluded such effects. Overall, it was remarkable how closely the mean external data logger (ambient) temperatures recorded were in comparison to the mean internal (body) data logger temperatures (Table 5), lending strong support to the use of data loggers at a scene to estimate body temperatures without the confounding effect of larval mass.

4. Conclusion

The focus of this paper was to determine the relationship between ambient temperatures (measured by dataloggers in sun, shelter and SS) and ‘bodies’ (pig heads) in a similar range of

environments. This study has clearly demonstrated the complexity of estimating accurate and precise temperatures experienced by insects feeding on a body, and the consequences of utilising inaccurate data for minPMI estimation models. Caution is advised regarding data logger placement at crime scenes, especially to measure the temperature of a body that has been lying in direct sunlight, when measurements of ambient temperatures, from unshielded data loggers or those in a SS, do not always reflect body temperatures. Although the placement of a data logger in a SS to estimate body temperatures showed no clear advantage over placing the data logger directly into the environment, it is recommended that a SS is used where possible and practical to comply with WMO recommendations [33]. For partially sheltered and sheltered bodies the differences between body temperatures and those of data loggers placed in the same situation are small; however, it is recommended that data loggers should be placed as close to and in as similar conditions as to where the body was found as possible, e.g., avoiding placement in front of windows if the body is shaded indoors.

The results of this study mark a necessary starting point for further research that is needed in this area to increase the accuracy of minPMI estimations, thereby leading to greater reliability of forensic entomology evidence presented in court.

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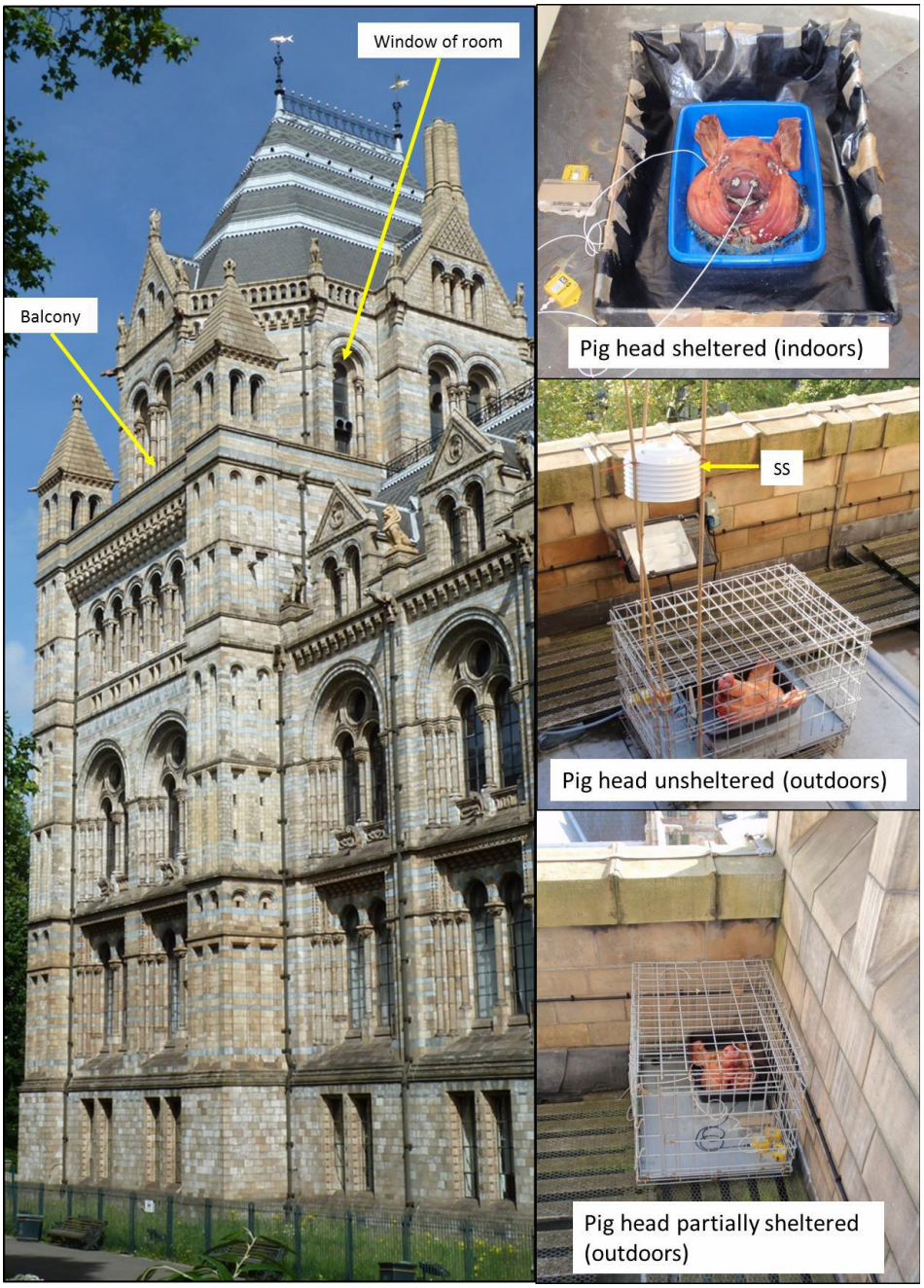


Figure 1: Three different experimental locations of adult pig heads during study on the southwest tower of the Natural History Museum, London, UK.

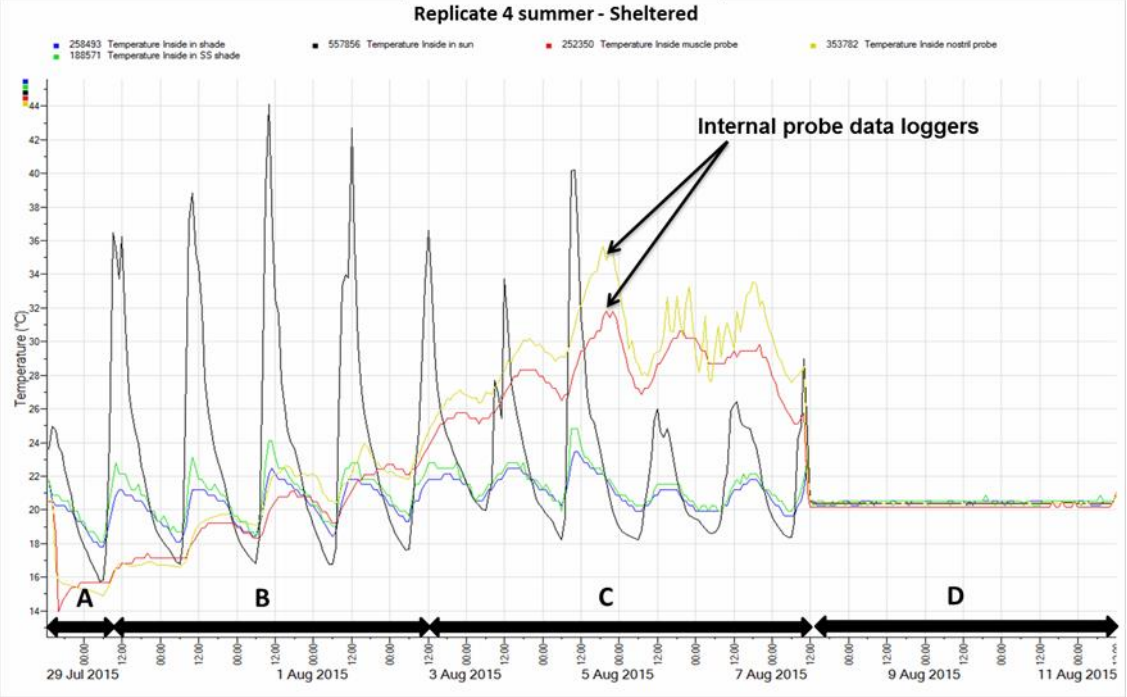


Figure 2: Overlay of 5 tinytag data logger records for Replicate 4 in the summer period, for the pig head in the sheltered location (indoors). The first 24 hours (Day 1) were termed the 'adaptation period', during which the temperature of the pig head adjusted itself to the temperature in the experimental location (A). From Day 7 onwards, a noticeable increase in temperature was observed for the two internal logger probes inside the pig head (C). To remove any confounding factors, only the 120 datapoints (=120 hours) of Days 2-6 were used for statistical analysis (B).
(Key: A, adaptation period; B, data range; C, larval mass heat effect; D, logger calibration in incubator).

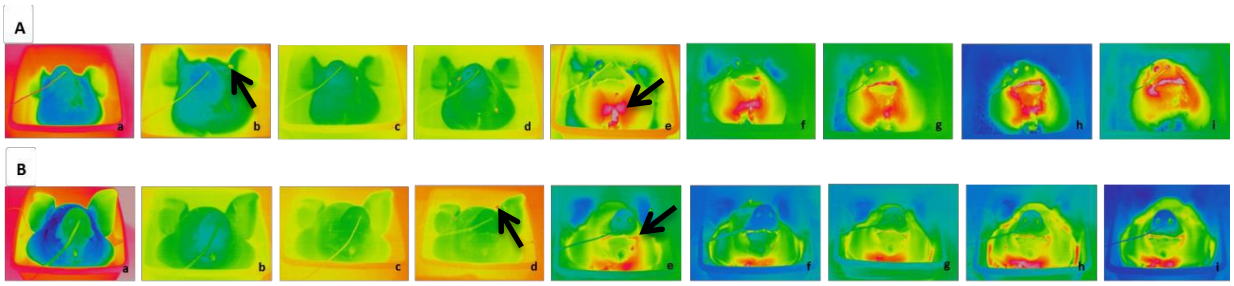
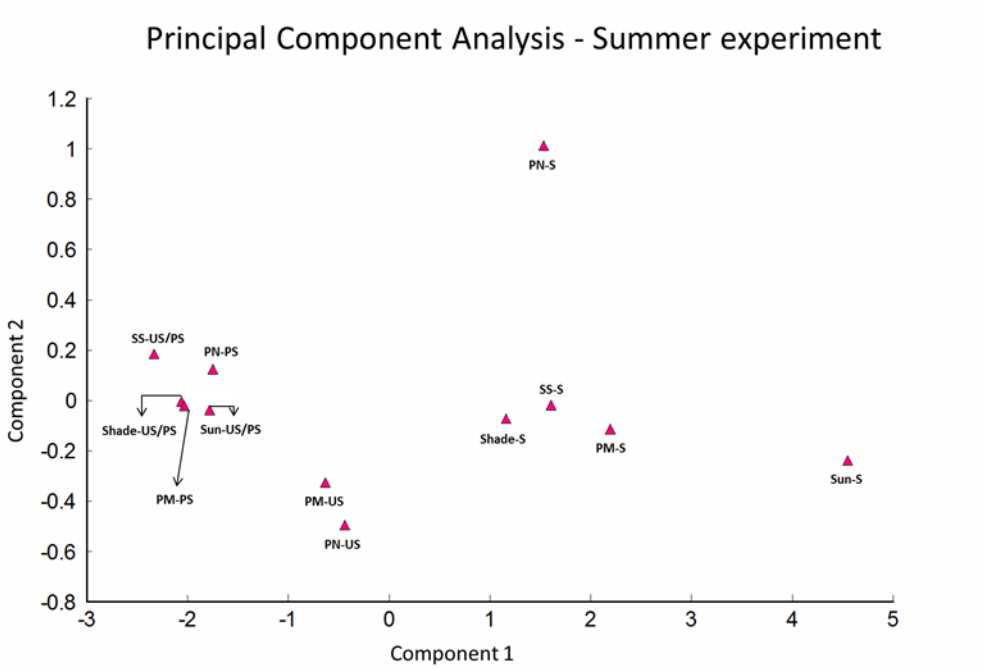
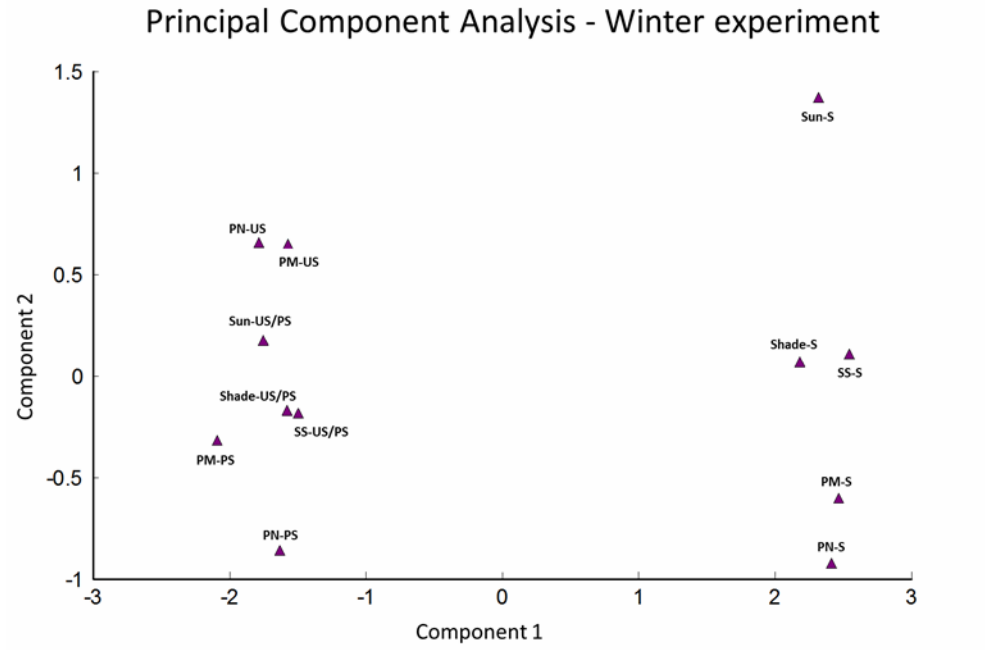


Figure 3: Thermal imaging NEC Avio Infrared Thermography H2640 camera frontal view images of summer (A) and winter (B) experiments of Day 1 – Day 4 (a – d) and Day 7 – Day 11 (e – i). **a** Adaptation period of 24 hours, where the pig heads adjust from cold storage temperature to ambient local temperatures. **b – d** First arrival of blow flies (warm spots - arrows A-b and B-d) on the heads. **e – i** First observation of larval mass in neck/mouth/nostrils area (arrows A-e and B-e) as well as increase of larval mass size over time. Temperature data for statistical data analysis was used from Day 2 – Day 6 only to exclude effects of storage and larval mass temperature interferences. The colours are not equivalent temperatures for each image due to different temperature ranges on the day of measurement, however the general rule of red = hot, blue = cold on a rainbow scale applies.





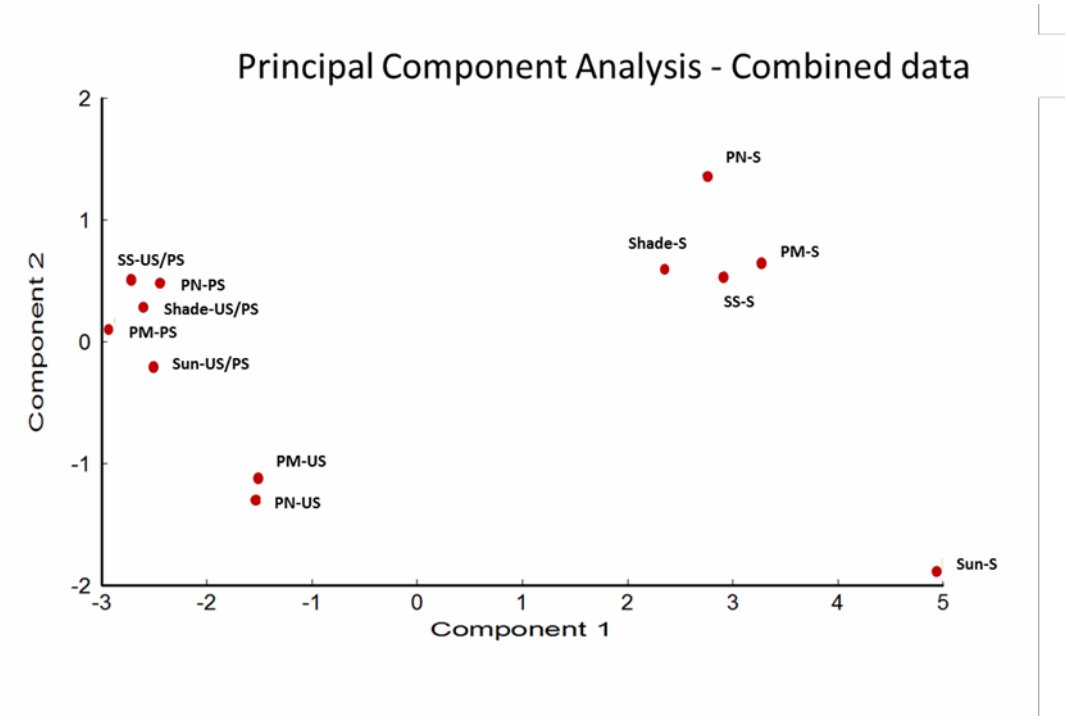


Figure 4: Principal Component Analysis (PCA) of the mean temperature measurements over five replicates for the three external data loggers surrounding the pig heads (in sun, in shade, in a SS) and the internal data logger probes inside the pig heads (in the nostril and in the muscle) for both summer and winter experiments (N=5), and for both datasets combined (N=10).

US=unsheltered pig head (outdoors), PS=partially sheltered pig head (outdoors), S=sheltered pig head (indoors).

PN=internal data logger probe in left nostril of pig head, PM=internal data logger probe in left cheek muscle of pig head, Sun=external data logger not sheltered from the environment surrounding a pig head, Shade=external data logger partially sheltered from the environment surrounding a pig head.

SS=external data logger sheltered in a Stevenson Screen from the environment surrounding a pig head.

Table 1: Positioning of Tinytag Plus 2® data loggers in and around the pig heads in three different environmental conditions.

Pig head unsheltered (outdoors)	Pig head partially sheltered (outdoors)	Pig head sheltered (indoors)
1 x logger sun (next to unsheltered pig head on ground)		1 x logger sun 10 cm next to SE facing window)
1 x logger SS (1.25m above unsheltered pig head in a SS)		1 x logger SS (1.25m above floor)
1 x logger shade (next to partially sheltered pig head on ground)		1 x logger in shade
1 x probe logger in left cheek muscle	1 x probe logger in left cheek muscle	1 x probe logger in left cheek muscle
1 x probe logger in left nostril	1 x probe logger in left nostril	1 x probe logger in left nostril

SS=Stevenson Screen

Table 2: Mean daily lux (illuminance) of a total range at 10:00 hours measured over 81 days summer (replicates 3-5) and 129 days winter (replicates 1-5) for the unsheltered, partially sheltered and sheltered locations for both seasons.

Season	Average lux and total range		
	Unsheltered (outdoors)	**Partially sheltered (outdoors)**	**Sheltered (indoors)**
Summer	15,268 lux (5,030 – 66,600 lux)[a]	6,601 lux (2,740 – 10,630 lux)[c]	897 lux (320 – 1,710 lux)[e]
Winter	15,697 lux (1,030 – 64,100 lux)[b]	2,998 lux (390 – 7,550 lux)[d]	476 lux (150 – 910 lux)[f]

Means followed by different letters are significantly different, $p<0.05$ (t-test).

Table 3: Mean temperature (°C) differences (MTD) ±95% confidence intervals (CI's), including Bonferonni- corrected p-values, between the three external data loggers surrounding the pig heads (in sun, in shade, in a SS) and the internal data logger probes inside the pig heads (in the nostril and in the muscle) for both summer and winter experiments (N=5). For the purposes of this study, we consider that MTD differences of more than 0.5 °C will have forensic significance, i.e. resulting in meaningful errors in minPMI estimations. The p-value (*t*-test) significance limit is 0.05, below which the output measurements between two loggers become statistically significantly different.

Experiment	Data logger position		Sun	Shade	SS
Summer	US	PN	1.04±0.45°C*#	1.26±0.63°C*#	1.46±0.85°C#
		PM	0.90±0.58°C	1.13±0.78°C	1.33±0.96°C
	PS	PN	0.054±0.37°C	0.28±0.58°C	0.48±0.65°C
		PM	0.20±0.22°C	0.030±0.27°C	0.23±0.37°C
	S	PN	2.28±1.09°C*#	0.43±1.51°C	0.056±1.30°C
		PM	1.87±0.75°C*#	0.84±0.60°C	0.47±0.57°C
Winter	US	PN	0.00062±0.52°C	0.15±0.96°C	0.21±0.93°C
		PM	0.14±0.81°C	0.013±1.24°C	0.074±1.19°C
	PS	PN	0.037±1.02°C	0.19±0.88°C	0.25±0.83°C
		PM	0.28±0.62°C	0.43±0.21°C*	0.49±0.20°C*
	S	PN	0.081±2.36°C	0.010±1.17°C	0.30±1.25°C
		PM	0.047±2.14°C	0.14±0.63°C	0.17±0.74°C

US=unsheltered pig head (outdoors), PS=partially sheltered pig head (outdoors), S=sheltered pig head (indoors)
PN=internal data logger probe in left nostril of pig head, PM=internal data logger probe in left cheek muscle of pig head
SS=Stevenson Screen

#= detected forensic significance, no statistical significance
*=detected statistical significance, no forensic significance
*#=detected statistical and forensic significance

Table 4: Refined statistical index of model performance (*dr*-index) according to Willmott *et al.* [35], for assessment of the accuracy of logger performances in relation to each other. This index (dimensionless) ranges from -1 to +1, with the upper limit showing optimal performance and the lower limit showing poor performance.

Experiment	Data logger position		Sun	Shade	SS
Summer	US	PN	0.56	0.44	0.36
		PM	0.62	0.51	0.42
	PS	PN	0.91	0.81	0.73
		PM	0.92	0.92	0.85
	S	PN	0.094	0.62	0.66
		PM	0.26	0.65	0.81
Winter	US	PN	0.94	0.86	0.85
		PM	0.89	0.83	0.83
	PS	PN	0.88	0.86	0.86
		PM	0.92	0.90	0.89
	S	PN	0.68	0.73	0.69
		PM	0.72	0.88	0.83

US=unsheltered pig head (outdoors), PS=partially sheltered pig head (outdoors), S=sheltered pig head (indoors)
PN=internal data logger probe in left nostril of pig head, PM=internal data logger probe in left cheek muscle of pig head
SS=Stevenson Screen

Table 5: Means of mean temperatures (°C; N = 5 replicates of 120 hours) including their CI (95%), and mean absolute error (MAE) for all used Tinytag Plus 2® data loggers in both summer and winter experiments. MAE was calculated to assess an average magnitude of total error for each data logger, i.e. their precision, as according to Willmott and Matsuura, 2005. The higher the MAE, the less precise the measurement performance of the data logger.

Data logger position	Summer experiment		Winter experiment	
	Mean±CI	MAE	Mean±CI	MAE
PN (US)	20.35°C±1.80	0.19	11.92°C±4.38	1.58
PM (US)	20.21°C±1.81	0.19	12.06°C±4.64	1.67
Sun logger (US/PS)	19.31°C±1.88	0.19	11.92°C±4.09	1.48
Shade logger (US/PS)	19.08°C±1.77	0.13	12.14°C±3.76	1.36
SS logger (US/PS)	18.88°C±1.87	0.14	12.08°C±3.79	1.35
PN (PS)	19.36°C±2.12	0.12	11.89°C±4.12	1.48
PM (PS)	19.11°C±1.95	0.11	11.64°C±3.68	1.33
PN (S)	22.09°C±3.32	0.13	15.48°C±2.61	0.94
PM (S)	22.50°C±2.62	0.12	15.61°C±2.29	0.82
Sun logger (S)	24.37°C±2.31	0.28	15.57°C±3.91	1.41
Shade logger (S)	21.66°C±2.08	0.077	15.47°C±2.33	0.84
SS logger (S)	22.03°C±2.22	0.084	15.79°C±2.26	0.81

US=unsheltered pig head (outdoors), PS=partially sheltered pig head (outdoors), S=sheltered pig head (indoors)
PN=internal data logger probe in left nostril of pig head, PM=internal data logger probe in left cheek muscle of pig head
SS=Stevenson Screen