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REVIEW

# Temperature: the weak point of forensic entomology

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**Abstract**
Measuring temperature is a key factor in forensic entomology. While noting factors to consider for a posteriori temperature estimation, many studies lack detailed methods or general rules allowing their integration into insect development-time calculations. This article proposes tools for determining the adequacy of weather station temperature datasets versus the local temperature experienced by carrion breeders. The idea is to start from a local scale (i.e., the cadaver) and gradually move to larger scales: at each step, the temperature can be increased, decreased or smoothed by environmental or biological factors. While a one-size-fits-all solution is not feasible for a complex and sensitive issue such as forensic meteorology, this checklist increases the reliability of minimum post-mortem interval (PMImin) estimation and the traceability of the proposed assumption.

**Keywords** Larval development · Post-mortem interval · Calliphoridae · Maggot-mass effect · Reliability

## Introduction

Forensic entomologists use carrion-breeding insects to estimate a minimum post-mortem interval (PMImin). This method relies on calculation of the development time of immature stages of insects, which depends on temperature [1]. Thus, measuring heat inputs is a key factor in forensic entomology and is likely the most crucial parameter affecting the PMImin calculation [2]. In a reference article reporting standards and guidelines in forensic entomology, the board of the European Association for Forensic Entomology (EAFE) boldly noted that "to age fly larvae, it is essential to (...) accurately determine the temperatures to which the larvae were exposed during their development either on or off the body" and that forensic entomologists should "only use reliable temperature data to estimate a time of death based on the developmental stage of the insects" [3]. However, a posteriori determination of the exact temperature prevailing during insect development is extremely difficult, if not impossible [4–6]. Accordingly, building a temperature dataset often involves a balance between accuracy and reliability. The aim of this article is to provide a clear, rational and easy-to-use framework for achieving this goal. To this end, we summarize the pitfalls of temperature estimations, highlight approaches for focusing on data reliability and suggest a working method for determining the adequacy of a weather station (WS) dataset versus the local temperature experienced by carrion breeders.

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## Temperature and necrophagous insects

Several studies have shown that temperature is the most significant factor affecting the development of necrophagous insects [1, 7–9]. The growth rates of many species, especially blow flies (Diptera: Calliphoridae), can be visualized as an asymmetric s-shaped curve: the developmental speed is low to null at low temperatures, increases linearly at medium-range temperatures and slows down at high temperatures up to a lethal threshold [7]. Due to this sigmoid relationship, growth rates at higher temperatures have a disproportionate effect on overall growth. This rule, commonly referred to as the rate-summation effect, is a well-known potential pitfall in development-time calculations and reinforces the necessity of accurate datasets with frequent (hourly or daily) recordings of temperature [10–12]. Here, we propose a top-down approach to check and rate of potential bias and errors in temperature estimation (Fig. 1). The idea is to start from a local scale (i.e., the cadaver) and gradually move to larger scales. At each step, the temperature can be increased, decreased or smoothed (i.e.,

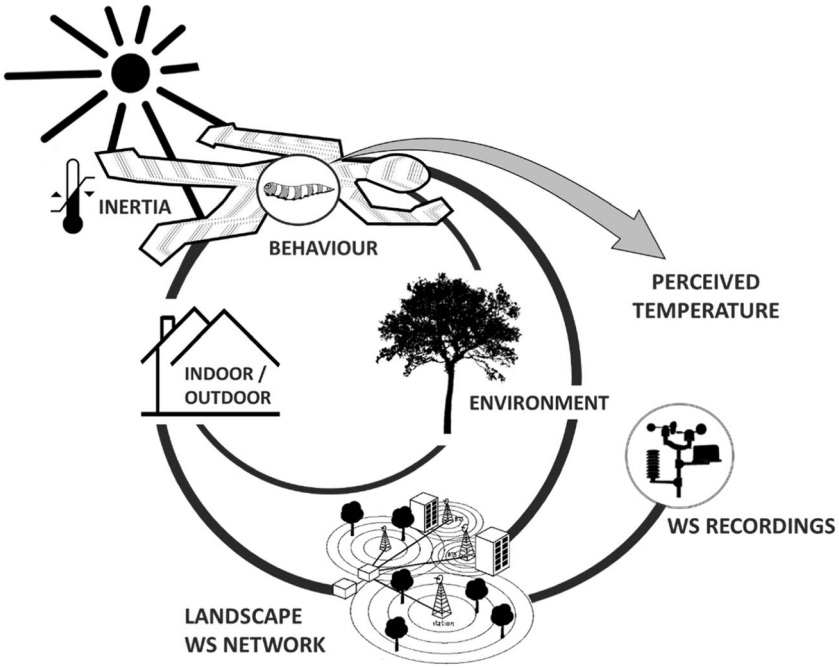


**Fig. 1** Schematic representation of the key elements affecting temperature in forensic entomology. On a local scale, cadaver thermal inertia and larval behavior affect the temperature perceived by larvae. The second circle shows the environmental factors affecting local temperature. The wider circle displays the weather and how temperature is recorded

mitigation of temperature changes) by environmental or biological factors.

## Cadaver cooling, thermogenesis and inertia

The cadaver itself affects the temperature experienced by larvae. Due to thermal inertia, death is followed by a period during which the body temperature progressively decreases [13]. This kinetic of cooling is affected by ambient temperature, body weight, clothing and environmental factors (e.g., wind and humidity). In all cases, eggs or young larvae laid on a corpse immediately after death will benefit the extra heat from the body. While the impact of body cooling usually falls within the confidence margin of the larvae development-time calculation, this impact must be considered for short post-mortem interval estimation based on first instars [14]. Thereafter, body temperature continues to react to ambient temperature changes with an inertia that mitigates abrupt changes by absorbing or emitting heat [15]. Accordingly, the temperature of a cadaver often differs from the ambient air temperature, and cadaver thermal inertia can affect larval development, especially during cold episodes [16]. Finally, in a few cases, aerobic bacterial metabolism can increase body temperature up to 10 °C above ambient temperature [17]. However, until proven otherwise, the effect of cadaver thermogenesis and inertia on larval development is likely negligible. As observed by Johnson et al. [17], maggots prefer to amass around the edges of carcasses, rather than directly within them, so the core temperature does not reflect the temperatures experienced by larvae (see next paragraph).

## Thermoregulation behaviour

During feeding stages, behavioral thermoregulation strategies allow larvae to optimize their growth [18]. As an example, Diptera larvae have been observed to feed on the warmer (sunny) sides of carcasses [19] or to enter the skull on cold nights, while moving back to the surface during the day [20]. Blow fly larvae also exhibit complex social behavior, including a constant trade-off between aggregation and thermal optimization [20, 21]. Large larval masses can indeed increase the local temperature by several tens of a degree and thus accelerate larval development (the so-called “maggot-mass effect”) [22–24]. Accordingly, when large masses (several thousands of larvae) are observed along with a significant increase in the recorded local temperature, the larvae may have developed at their maximum speed [25, 26]. However, inside such masses, larvae move away from locations that are too hot or overcrowded, resulting in constant turnover [27]. As a result, individual larvae experience continuous variations in temperature during their development. Therefore, precise and reliable determinations of the temperatures experienced by larvae growing within a large aggregate are impossible [28]. In such a case, the usual recommendation is to determine their minimum (maggot-mass effect) and maximum (ambient temperature) development times and use the resulting interval to estimate PMImin [9].

## Surrounding environment

Local parameters (i.e., the surrounding environment of the cadaver) also impact the temperature perceived by

necrophagous larvae. First, the indoor/outdoor location of a corpse strongly affects cadaver accessibility, the decomposition process, the insect species involved and, obviously, the temperature during decomposition [29]. Indoor locations are often characterized by temperature control systems (heaters and/or coolers) and lower amplitudes of temperature changes (thermal inertia). However, even inside dwellings, temperature fluctuates due to thermostatic regulation or sun exposure. Therefore, ambient temperature is never constant, and a temperature interval, rather than an inevitably inaccurate single value, should be considered. Furthermore, the occurrence of insects on a corpse is often congruent with the presence of openings (e.g., a partially opened window) affecting the inside temperature unpredictably [30, 31]. In such cases, temperature recordings are required to determine the correlation between outdoor and indoor values.

Regarding outdoor locations, vegetation, especially large foliated trees, creates a shaded environment, significantly decreasing peak temperatures during summer. Conversely, obstacles such as twigs or waste that are used to cover/hide a cadaver will block wind and enhance local thermal inertia [32]. The position of the cadaver in its environment must also be considered. A corpse lying on the ground will be close to ground temperature, while a hanged body may be closer to the ambient air temperature [32]. Therefore, considering how WS data are recorded and the types of values that are optimally matched is especially important. Regular WS temperature values are recorded under a shelter 1 m above the ground surface. However, the most complete and stringent WS recording often include measurements of open air and inside ground temperatures at different heights/depths. Working with daily means adds even more complexity, as average values can be calculated as the sum of hourly values divided by 24 h or as the mean of the daily minimum and maximum.

## Weather station network, temperature recordings and a posteriori estimation

Because of their particular geographical locations, different WSs will not record the same temperature values. In the best-case scenario (this is not necessarily the usual case), the forensic entomologist can rely on regional or national WS networks and a forensic climatologist to select a relevant station. One important parameter is the distance to the location at which the corpse was discovered, but the height (altitude) of the station and wind exposure must also be considered [31, 33, 34]. Furthermore, the closest WS may have a poor sampling resolution (e.g., only daily mean values or a restricted number of climatic parameters may be available) compared to a farther but more sophisticated station (synoptic stations are fitted with various standardized instruments and collect information at least every 3 h). To make a rational, objective choice between these stations, the usual recommendation is to record the on-site temperature (i.e., the temperature at the location at which the cadaver was discovered) for a few days/weeks and then backcheck the recordings of the closest matching WS [2, 3]. However, the criteria for determining the most relevant station are unclear. For instance, the best match may differ between days and climatic conditions, or the data of a given station may fit perfectly except for some extreme values [35]. Therefore, even with adequate statistical tests, making an informed decision regarding the relevance of data from a WS is tricky.

The correlation between local temperature and WS values can also be modeled. These mathematical models can subsequently be used for a posteriori correction of the WS recordings. Several authors recommend this method to obtain estimated local temperature values, arguing that the corrections allow forensic entomologists to accurately consider the local microclimate [2, 3, 36]. However, this solution has a disputable scientific basis and uncertain benefits [35, 37]. Clearly, if one could easily and accurately determine the temperature in a given location from data collected in another location, the WS network would be useless, and microclimatology would not exist [38]. In fact, very practical considerations, such as the exact location of the temperature recorder, the setup used to protect the recorder or correlation periods can strongly affect the accuracy and reliability of the measurements [4, 35]. Furthermore, different studies have demonstrated potential bias and risks of errors resulting from a posteriori temperature estimates [4, 35, 37]. Within this context, the forensic entomologist should examine each situation to determine whether the use of an estimated temperature is truly appropriate. This choice should be made according to (1) the necessity of providing a definite larval development time, rather than a time interval, (2) the quality of the correlation models that can be used, and (3) technical feasibility (e.g., the availability of temperature recorders, processing times, access to the crime scene).

Table 1 summarizes the factors listed in this review as a qualitative checklist. For a given case, this table can be used to quickly verify the factors affecting local temperature. Key references are proposed and can be consulted for more details on each point. The reader must refer to the algorithm below when several factors act together (e.g., the presence of a large maggot mass feeding on a concealed cadaver located outdoors with sun exposure).

## A qualitative method for assessing temperature effects in forensic entomology

Based on this review, we propose an algorithm to prioritize and quantify multiple effects that may impact the local temperature in forensic entomology analysis. For the sake of clarity, references have not been included (they can be found in the review portion of this article). The main principle is to start from local conditions (i.e., conditions on the cadaver) and progressively move to larger scales: one example is provided

**Table 1** Qualitative checklist of factors potentially increasing, decreasing or damping the temperature perceived by necrophagous larvae during their development. Factors applying only while insects are on the cadaver are indicated in italics, and factors occurring only during feeding stages are indicated in bold italics

| | | T°C INCREASE | T°C DECREASE | DAMPING |
|---|---|---|---|---|
| LOCAL | LARVAL MASSES | ***Up to tens of °C above ambient - limited to the species-specific upper threshold [22, 23]*** | | Limitation of T°C decrease [16] |
| | THERMAL REGULATION BEHAVIOUR | ***Larvae as close as possible to the species-specific optimum dev. T°C [21, 24]*** | ***Larvae remaining above the specific threshold [21]*** | ***Larvae optimizing T°C (as close as possible to the species-specific optimum) [21, 24]*** |
| | BODY COOLING | *Only significant for short-term PMImin estimation based on first developmental instars [13, 14]* | | |
| | CADAVER THERMAL INERTIA & THERMOGENESIS | *Heating due to bacteria. Likely negligible (restricted to aerobic decomposition & inner parts of carcasses) [17]* | | *Smoothing of ambient T°C changes - varying according to body mass, clothes, etc. [13, 32]* |
| | CONCEALMENT | | | Smoothing of ambient T°C changes - varying with concealment conditions [13] |
| SURROUNDINGS | INDOORS | Can be important with functional heating | Can be important with air conditioning | Low with openings, high with heating/air conditioning [31] |
| | SUN EXPOSURE | Direct sunlight: T° increase during daytime, but most larvae avoid direct sun exposure. [19, 38] | Only in shaded areas. Limited if reference weather station data have been collected under a shelter [35] | |
| | WIND EXPOSURE | | Usually, low except in constantly windy areas (e.g., seashores) [38] | |

| |
|---|
| Low impact, usually limited to 1-2°C max. |
| **Medium impact, can sometimes exceed 5°C** |
| **High impact, can exceed 10°C** |

at the end. Essentially, the emphasis is placed on reliability, which implies the use of a margin of error and can result in a lack of accuracy.

All temperature corrections related to local effects (see Table 1 and Fig. 1) must be applied only to feeding instars growing on the corpse, not to wandering larvae or remote pupae.

(1)

If only very young (eggs, first or second instar) larvae are present, a short PMImin estimation can be determined based on the development of these samples. The possibility extra heat resulting from body cooling should be considered for the development-time calculation.

Otherwise, proceed to step 2.

(2)

If Diptera larvae are aggregated in a large mass (several thousands of larvae), and/or a significant local temperature increase is recorded inside larval aggregates (several degrees above ambient temperature), and/or several thousand pupae are found, larvae may have benefited from extra heat due to maggot-mass effect. For each species, consider the minimum development time during the feeding instar. Continue to step 3 to calculate the maximum development time.

If only a few larvae or pupae are present, the ambient temperature was always low (below 10–15 °C) during larval development, or no significant heating is recorded inside the maggot masses, proceed to step 3.

(3)

If the cadaver is located indoors, proceed to step 4.

If the cadaver is located outdoors, proceed to step 5.

(4)

If temperature regulation setups and a thermostatic programmer are in operation, use the set temperature(s) with a ± 1 or 2 °C margin, which will result in three values that provide a likely estimation and margin of error. The temperature margin must be extended in cases of small openings (e.g., a slightly opened window in an adjacent room).

If no functional heating/air conditioning and/or large openings are present (e.g., a broken window in the same room as the cadaver), consider the average indoor and daily outdoor temperatures to obtain the egg-laying interval (see step 5 for the choice of the WS and data recordings).

(5)

If conditions involve an open area during the warm season with high sunlight, then the local temperature likely differs from WS recordings. Accurate and rational determination of heat imputs and how larvae reacted (e.g., larvae moving under the body at the interface with the soil to avoid direct sun exposure) is currently not possible. Consider that feeding larvae may have benefited from sun warming and reached their maximum development speed. Hourly temperatures or an hourly based daily average can be used to calculate the minimum larval development speed (step 6). These two values will provide the minimum and maximum feeding larva development times. Proceed to step 6 for the temperature during post-feeding instars (i.e., wandering larvae and pupae).

In any other case, proceed to step 6.

(6)

If a nearby WS can provide hourly or daily temperature values recorded under a shelter, the correlation between WS data and local temperature can be assessed using a posteriori local recordings. If there are no significant differences between WS recordings and the local temperature, then the WS values can be considered representative of the local temperature prevailing during development. If the cadaver is highly exposed to temperature changes (e.g., in conditions involving thin clothes, a hanged body, or strong winds), hourly temperatures (or hourly based daily averages) will reflect promptly changes in the local temperature. If the body is thermally insulated (e.g., in conditions involving warm clothing, wrapping or concealment under a thick layer), daily average values (Tmin+Tmax/2) may be more appropriate to reflect the thermal damping effect (Table 1).

If no close or similarly exposed WS is available, or if there is uncertainty, then the local temperatures recorded by dataloggers over several days can be used to apply a posteriori corrections. However, this method is subject to strong restrictions and has uncertain benefits. A margin of error can also be created using several WS datasets. In any case, the risks of errors should be carefully considered and clearly assessed, and the methods used must be adequately reported in the conclusions.

To highlight the use of this algorithm, a case example is provided here. A few third-instar *Lucilia sericata* were sampled on a corpse that was concealed with twigs. The cadaver was discovered during summer in a sun-exposed wasteland. In this case, several parameters may affect the local temperature and larval development (Table 1). However, these factors do not have the same effect or intensity: while sun exposure has the potential to increase local temperature, concealment can have a moderate damping effect on sun heating and temperature changes. Application of the above algorithm suggests that larvae may have reached their maximum development speed (sun warming, point 5). Accordingly, the minimum development time of *L. sericata* should be regarded as a possibility. However, larval sun avoidance and thermoregulation behavior must also be considered (point 5). For this purpose, WS temperature recordings can be used to calculate the minimum larval development speed. Due to the concealment of the cadaver, the use of daily average values (Tmin+Tmax/2) to reflect the thermal damping effect of the twigs seems to be the better option (point 6). These calculations will result in an egg-laying interval that will include the actual egg-laying event.

## After cadaver removal

After the discovery of a body and on-site sampling of insects, the forensics team must ensure proper conservation and transport of the sample and the traceability of its thermal history. In practice, many errors or biases occur during this time lapse [16, 39, 40]. The main pitfalls and some suggestions to avoid them are listed below.

According to the usual standards and guidelines of forensic entomology, insects should be sampled on site before corpse removal and later at the morgue or during autopsy [2, 3]. Subsequently, living samples must travel from the site to the morgue and laboratory. Ideally, transport is achieved quickly and under controlled temperature conditions; however, transport during hours with unknown temperatures often occurs. While the consequences for PMImin estimation are typically negligible and fall within the margin of error, it is important to inquire about whether extreme values occurred. Indeed, temperatures in refrigerated transports may locally fall above zero, and sun-exposed or overcrowded sample bags can reach lethal temperature thresholds. Upon arrival at the forensic institute or laboratory, corpses and samples are refrigerated until analysis/autopsy. This storage period can extend to several days and sometimes weeks, making this period of time an important source of possible error [16, 39–41]. Indeed, the refrigeration temperature is rarely precise or strictly controlled, and deviations are difficult, if not impossible, to trace. For example, the temperature inside a regular refrigerator, such as those used in many police stations to preserve samples, can deviate by several degrees according to the location of the sample inside the refrigerator, thermostat settings, the cooling process, and door opening. To overcome this problem and ensure thermal traceability, temperature recorders can be attached to samples. Regrettably, this solution is still underused [42]. Therefore, the use of a margin of error (e.g., refrigerator temperature = 4 ± 2 °C) is strongly recommended.

Once received at the forensic entomology laboratory, living larvae are usually bred until adults emerge [3]. Because a significant part of insect development may occur in the laboratory, the temperatures inside rearing chambers must be strictly controlled and recorded relying on high-quality equipment, efficient metrology and certified calibration. Nevertheless, consideration of the inherent limitations of the materials is important. For example, a new top-range climatic chamber (Panasonic MIR 554) exhibits thermal fluctuations of ± 0.2 °C (heating) to ± 1.5 °C (cooling) and temperature uniformity of ± 0.5 °C. Thus, the recommendation is also to add a margin (at least 1 °C) to the chamber-set temperature.

In some cases, fixed site samples can be used as an internal control [42]. Indeed, the egg-laying dates calculated for larvae sampled and fixed on site should match those obtained for insects sampled and/or bred at the laboratory. For each species, PMImin calculations should overlap; discrepancies between these two sample sets may indicate problems during transport/conservation/breeding or an invalid thermal history during these steps.

## Conclusion

While several of the concerns presented in this article have already been analyzed in research reports, such studies often lack detailed methods or general rules allowing integration of their results into development-time calculations. In an article entitled "Using estimated on-site ambient temperature has uncertain benefit when estimating postmortem interval", [35] concluded that "Despite the fact that WS data are likely to be different from conditions experienced at the crime scene, they should be considered, by default, as the most ideal data to use for PMI estimations (...) as long as (deviations) are taken into account when providing PMI estimations and an appropriate range is given." This quote clearly demonstrates the necessity of guidelines and standards to clearly identify and estimate these deviations and their impacts on PMImin estimation. While a one-size-fits-all solution is not feasible for a complex and sensitive issue such as microclimatology, the process described in this article at least provides a clear framework for forensic entomology and allows traceability of the proposed assumption. However, although the reliability of PMImin estimation is stronger, the use of a temperature interval as recommended will result in a less accurate result. We believe that this outcome is preferable to a misleading impression of overall accuracy and precision.

## Compliance with ethical standards

**Competing interests** The authors declare that they have no conflict of interest.

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