Low temperature episodes in development of blowflies: implications for postmortem interval estimation

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Abstract. Traditionally the calculation of accumulated degree days or hours (ADD or ADH) involves the concept of a minimum threshold temperature below which development ceases. Hence in fluctuating conditions, where temperatures drop below this threshold, there may be periods of time when development is taken to be zero. This has important implications when the calculation of post-mortem interval (PMI) is based on the ADD or ADH of larval dipterans. Normal development of larvae of the blowflies Calliphora vicina Robineau-Desvoidy and C. vomitoria L. (Diptera: Calliphoridae) at 20°C was interrupted by cold episodes. The expectation was that total development time would increase by the period at low (therefore no development) temperature but the total ADD or ADH should be the same as non-cold treated cohorts. The results, however, showed that total ADH for both species decreased linearly with increasing temperature with no evidence of any minimum threshold temperature effect. The increased ADH at low temperatures may be due to either continued but reduced development or a delay in development restarting after the cold episode. Use of ADH in PMI estimations has shortcomings particularly during the winter period where low temperatures are involved or where there are sudden summer cold spells during the development period. As blowfly development progresses from egg to pupa such errors will be compounded.

Key words. Calliphora vicina, Calliphora vomitoria, development threshold temperature, forensic entomology, postmortem interval.

Introduction

In forensic science, insect evidence is most commonly used to estimate the time of death of a corpse (the postmortem interval, PMI). Some of the first insects to invade a corpse in the U.K. are Calliphoridae (Diptera), including species of Calliphora, Protophormia and Lucilia (Lane, 1975). In many temperate regions Calliphora species are considered the most important, with Calliphora vicina (Robineau-Desvoidy) (= C. erythrocephala Meigen) and Calliphora vomitoria (Linnaeus) being widely distributed throughout Northern Europe. In favourable conditions these species lay their eggs around natural orifices or wounds on the fresh corpse within a short time after death. These hatch to larvae, which grow through three stages. During the latter part of the third stage, larvae stop feeding, migrate from the corpse and burrow down into the soil to pupate. The pupal stage has the largest duration before adults emerge.

Being poikilothermic, the rate of development in insects is governed by ambient temperature; the higher the temperature the faster development occurs. Thus by knowing the ambient temperature and the progress of blowfly development, an estimation of time since the eggs were laid can be made.

Two methods to estimate PMI using blowfly development are available. Larval size, usually length, can be compared with data on larval size related to temperature

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and time in an isomegalendiagram (Grassberger & Reiter, 2001). There are a number of difficulties with this approach, including the actual size, the limited number of published isomegalendiagrams and the restriction of this method to the larval stages only. The other approach is to measure the accumulated degree days or hours (ADD or ADH) needed to reach a particular stage of development. Accumulated degree days or hours are the product of temperature above a species’ (and possibly geographical population) minimum developmental threshold and the time spent at that temperature. Prediction of insect development focuses on the range where the relationship between development rate and temperature is constant and is based on the work of Abrami (1972), Allen (1976), Arnold (1960), and Baskerville & Emin (1969) (see also Wagner et al., 1984). One difficulty with this linear day/hour degree model is that it only truly applies to the thermal range where temperature is directly proportional to development. The calculated ADD/ADH required for development will be too low at temperatures near the lower threshold and too high near the optimum temperature (Wagner et al., 1984). Additionally, although it is accepted that development ceases at low temperatures (Laudien, 1973 cited in Lamb et al., 1984), the lower threshold temperature is often very difficult to determine accurately because insects survive for long periods with near zero development. The Development Threshold Temperature (DTT) is most commonly estimated by measuring developmental rates at a range of temperatures and fitting a regression line to the results. This can then be extrapolated to the x-axis where development is zero.

According to Higley & Haskell (2001) who reworked Kamal’s data (Kamal, 1958), the DTT for C. vicina and C. vomitoria is 6°C, yet recent studies suggest this varies with locality (Saunders & Hayward, 1998). The majority of past research on the effects of temperature on development on species in the Calliphoridae has been done at temperatures close to the optimum, mainly over 20°C (Dallwitz, 1984; Greenberg & Tantawi, 1993; Anderson, 2000). Vinogradova & Marchenko (1984), however, did include lower temperatures. In Britain and Northern Europe, temperatures for the greater part of the year are relatively low. Calliphora vicina and C. vomitoria are able to develop at these low temperatures and C. vicina is active through the year.

This study reports on a series of laboratory experiments that explore the effects of short periods of low temperature on the development of two locally common blowfly species with particular reference to ADD/ADH estimation. A recent study (Myskowiak & Doums, 2002), considering the impact of the practice of refrigeration of insect evidential material (using Protophormia terraenovae Robineau-Desvoidy) to temporarily halt development until passed to a forensic entomologist, is complementary to this present study.

Materials and methods

Wild populations of C. vicina and C. vomitoria were raised and maintained in gauze covered cages (22 x 38 x 27 cm) at room temperature (20–22°C). Flies were supplied with granulated sugar and water ad libitum. New flies were added to these populations as the experiment progressed. Each cage only contained approximately 100 flies to avoid overcrowding (Saunders, 1997).

A few days before eggs were required, liquid liver exudate was provided as a protein source for the flies. Fresh pigs’ liver was then introduced as an oviposition and larval food source. Females massed upon the liver and oviposition would normally begin 30 min to 1 h later. The time of oviposition was noted and the liver and eggs were removed from the adult cage. Eggs were then separated onto new liver in a disposable weighing boat. To prevent any larval mass effects which might cause a localized temperature elevation (Vinogradova & Marchenko, 1984 cited in Catts & Goff, 1992; Turner & Howard, 1992), eggs were separated into small clumps of about two to five and spread over the liver. The liver, which had been kept refrigerated, was allowed to warm up to room temperature before the eggs were placed on it. Approximately 30–40 eggs were used in each experimental replicate.

The weighing boats, containing liver and eggs, were placed on a 2-cm layer of soil-less compost in individual plastic containers (7 x 8 x 11 cm) with a plastic lid. The compost provided a medium in which the post-feeding larvae could pupate. The liver was changed regularly so that food for the larvae was always in excess, to avoid competition. The containers were then kept at 20°C (Cooled Incubator, LMS, Sevenoaks, Kent, U.K.) and a second incubator (Cooled Incubator, Sanyo-Gallenkamp, U.K.) was used when needed for the alternative temperature. The laboratory was air-conditioned at 20°C ± 1°C so that the removal of larvae from the 20°C incubator for measurement did not vary their temperature regime. The second incubator (monitored using a Tiny Tag, Gemini data loggers, Chichester, U.K.) returned to the set temperature within 10 min of opening and closing the door to put in the experimental larvae and was then left unopened until the end of the exposure period. Larvae were kept in continuous dark. Statistical analyses were carried out using the SAS package STATVIEW v. 5.01.

The effects of low temperature episodes were explored in two series of experiments on both blowfly species. Specific criteria for each series were influenced by previous experimental experience. Development was followed from the egg stage through to adult eclosion.

Series 1 – Low temperature period at different development stages

Egg development took place at 20°C. Individual cohorts of larvae were then subjected to cold episodes, of 5°C for 5 days, at one of the following developmental stages – larval stages one, two or three (feeding) or the pupal stage. Following the cold episode the blowflies were returned to 20°C for the remainder of their development. Controls remained at 20°C for the entire developmental duration.
Series 2 – Differing low temperatures for a constant time period

All experiments were kept at 20°C for 3 days following oviposition. By this time the larvae were late second stage. Cohorts of these larvae were placed at 1°C, 3°C, 5°C or 10°C for 5 days, after which they were returned to 20°C for the remainder of development. Again, controls remained at 20°C for the entire developmental duration.

Three replicate cohorts, each starting with approximately 30 eggs, were individually followed in each treatment within an experimental series. Following oviposition, each cohort of eggs was checked half hourly, until all were hatched. Subsequently they were observed every 2 h between 10.00 hours and 18.00 hours each day. Different starting times were used to co-ordinate hatching and metamorphosis events with the daytime observational periods. Immature stages were examined under dissecting microscope to determine developmental stage. On pupariation, the puparia were removed from within the compost and placed upon the surface. As the adults emerged, they were counted and removed from the containers. During all experiments, the time in hours since oviposition when the insects progressed from one stage to another was recorded.

To calculate the ADH from the developmental time the following formulae was used:

\[
\text{ADH} = \left( \text{development time (h)} - \text{time in cold episode (h)} \right) \times 20 + \left( \text{time in cold episode} \times \text{temperature of cold episode (°C)} \right).
\]

As a result of the uncertainty of the concept of a DTT, initially no correction for this was applied to any of the data for either species.

Results

Development at 20°C – the controls

Each of the experimental series included a set of controls at 20°C. The duration and cumulated values for the control data have been combined in Table 1. At 20°C, the timings for the egg stage and 1st and 2nd stage larvae are quite similar for C. vicina and C. vomitoria. The 3rd stage larvae period for C. vomitoria takes about 50% more time than for C. vicina, whilst the pupal stage is some 12% shorter (Fig.1). Thus overall the total development time for C. vomitoria is 34 h longer than C. vicina, 683 more ADH, assuming both have a DTT of 0°C.

Experimental series 1

Excluding the controls, each treatment had a 5-day period at 20°C (≈ 2400 ADH) replaced by a 5-day cold episode of 5°C (≈ 600 ADH). The linear hour-degree model suggests that insects need to have accumulated a specific number of degree hours to move from stage to stage. Therefore, regardless of where the cold episode occurred, whilst the developmental time will be longer (to make up the deficit of 1800 ADH), the ADH value to reach a specific stage should not be significantly different from the corresponding control ADH value.

This was not the case for the ‘post cold episode’ stages of either species (Fig.2). Bartlett’s test for homogeneity of variances among treatments indicated that the group variances were not equal for either blowfly species (C. vicina \( F = 8.3, P < 0.001 \); C. vomitoria \( F = 10.1, P < 0.001 \)). As this renders the standard one-way ANOVA inappropriate, Welch’s one-way ANOVA test for data containing groups with unequal variances was used instead. This showed that there are significant differences between the treatments for both species (C. vicina \( W = 102.5, P < 0.001 \); C. vomitoria \( W = 46.5, P < 0.001 \)). This is seen in Fig. 2 by comparing the Control ADH values with the treatment ADH values where there is a difference of approximately 600 ADH, suggesting that no development took place in either species during the 5°C period: that is the DTT is around 5°C.

The data were reanalysed using 5°C as the DTT. Welch’s test, applied to the recalculated ADH values in each treatment, showed that for both species there were significant differences between ADH values (C. vicina \( W = 3.2, P = 0.016 \); C. vomitoria \( W = 2.7, P = 0.033 \)). For C. vicina, the developmental stage at which the cold episode occurs appears important. Scheffé’s post hoc test (Table 2) shows a significant difference only between the 1st stage larva and pupal treatments, but low \( P \)-values between the 1st stage larval treatment and all the others for C. vicina.

This effect is not seen in C. vomitoria. Scheffé’s test shows no significant differences between the treatments in this species. The ADH values to reach adult emergence are all similar to each other (\( P \)-values all > 0.1) regardless of when the 5-day cold episode occurs in the developmental period.

Experimental series 2

As the temperature of the 5-day cold episode increased, the development time (in hours) to reach the adult stage for both species decreased linearly (Fig.3). Both slopes are significantly different from zero (Fig.3). There was an increase of approximately 8 h on the development time for each °C decrease in the 5-day cold episode.

If the DTT is 5°C (based on Experimental series 1), we would expect there to be no significant difference in the developmental times for the 1, 3 and 5°C data. However, a significant difference between the development times for each temperature was seen using Welch’s test on the data sets of each species (C. vicina \( W = 2027.2, P < 0.001 \); C. vomitoria \( W = 1529.4, P < 0.001 \)). Using Scheffé’s multiple comparison test on these data indicated significant differences (\( P < 0.001 \)) between all temperature combinations in both species. This appears to be at variance with the findings of the first experimental series (although this was limited to only one temperature for the cold period) and...
Table 1. Mean duration, standard deviation, range and accumulated degree hours of the egg, larval and pupal stages of *Calliphora vicina* and *Calliphora vomitoria* at 20°C.

<table>
<thead>
<tr>
<th>Stage</th>
<th><em>C. vicina</em> Mean duration of stage (h)</th>
<th>Mean cumulative duration (h)</th>
<th>Cumulative duration min–max</th>
<th>ADH</th>
<th><em>C. vomitoria</em> Mean duration of stage (h)</th>
<th>Mean cumulative duration (h)</th>
<th>Cumulative duration min–max</th>
<th>Cumulative duration min–max</th>
<th>ANOVA of ADH C. vicina vs. C. vomitoria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>160</td>
<td>24.7</td>
<td>24.7</td>
<td>1.3</td>
<td>21.8–22.8</td>
<td>493</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larval 1</td>
<td>97</td>
<td>17.9</td>
<td>42.5</td>
<td>4.9</td>
<td>36.3–50.5</td>
<td>851</td>
<td></td>
<td></td>
<td>**</td>
</tr>
<tr>
<td>Larval 2</td>
<td>104</td>
<td>44.1</td>
<td>86.6</td>
<td>5.7</td>
<td>74.0–119.0</td>
<td>1733</td>
<td></td>
<td></td>
<td>**</td>
</tr>
<tr>
<td>Larval 3</td>
<td>140</td>
<td>123.4</td>
<td>210.0</td>
<td>12.9</td>
<td>179.0–252.8</td>
<td>4200</td>
<td></td>
<td></td>
<td>**</td>
</tr>
<tr>
<td>Pupae</td>
<td>106</td>
<td>328.5</td>
<td>538.4</td>
<td>12.5</td>
<td>501.0–563.0</td>
<td>10769</td>
<td></td>
<td></td>
<td>**</td>
</tr>
</tbody>
</table>

*Significant at 1% significance level.*
suggests there is no clear cut minimum development threshold for either blowfly species within the temperature range covered in these experiments (1–20°C).

Rather than the expected consistent ADH values, across the range of temperatures (that is an expectation of gradient \( b = 0 \)), adult ADH values for both species follow a decreasing linear relationship with increasing temperature for the 5-day cold episode, which are significantly different from zero (Fig. 4).

Using Welch’s test on each species’ ADH and temperature data set (and taking the DTT point to be 5°C) reveals that, for both species, there is a significant difference between 5-day cold temperature episodes and ADH values

\[ \begin{align*}
\text{(a)} \quad & C. \text{vicina} \\
& 24.65 \quad 17.89 \quad 44.1 \quad 123.36 \\
\text{(b)} \quad & C. \text{vomitoria} \\
& 23.62 \quad 15.53 \quad 47.23 \quad 195.5
\end{align*} \]

*Fig. 1. Comparison of relative proportions of the egg, larval and pupal stages to the total developmental time in Calliphora vicina and Calliphora vomitoria life cycles. Figures in the pie charts are the mean duration of each developmental stage in hours at 20°C.*

\[ \begin{align*}
\text{Control} & \quad 12000 \\
\text{Larval 1} & \quad 11000 \\
\text{Larval 2} & \quad 10000 \\
\text{Pupa} & \quad 8057 \\
\text{stage} & \quad 8549
\end{align*} \]

*Fig. 2. Boxplots of total ADH against the stage at which cold episode (5°C/5 days) occurred. (a) Calliphora vicina, (b) C. vomitoria. *Indicates outliers. The line across the box is the median. The bottom of the box is at the first quartile (Q1), and the top is at the third quartile (Q3) value. The lines that extend from the top and bottom of the box show the range of the distribution.*

\[ \begin{align*}
\text{Larva 1} & \quad 0.050 \\
\text{Larva 2} & \quad 0.009 \\
\text{Larva 3} & \quad 0.088 \\
\text{Pupa} & \quad 0.973 \\
\text{Control} & \quad 0.960
\end{align*} \]

\[ \begin{align*}
\text{Larva 1} & \quad >0.999 \\
\text{Larva 2} & \quad >0.999 \\
\text{Larva 3} & \quad 0.886 \\
\text{Pupa} & \quad 0.723 \\
\text{Control} & \quad 0.175
\end{align*} \]

Table 2. Scheffé’s multiple comparison significance table between total ADHs for differing treatments of cold episodes (5°C/5 days) during different life-cycle stages for Calliphora vicina (top right) and Calliphora vomitoria (bottom left). Bold indicates significant at the 5% level.

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As the DTT value was set at 5°C, the impact of the 5-day period at 1 or 3°C should have had no numerical input to the ADH calculation. However, the data clearly shows that the development rate outside the cold episodes was affected by the low temperature experience. The cold episodes below the DTT had a negative influence, having some unknown effect, delaying development, resulting in an increased total ADH.

The impact of the level set for the DTT on the ADH estimate can be seen in Fig. 5. Two things are evident. As a result of the mathematics of calculating ADH, changing the DTT value affects the controls at 20°C because the hourly multiplier is the difference between the DTT and control temperature. Thus lowering the DTT from 5 to 0°C increases the hourly multiplier from 15 to 20. Lowering the minimum threshold also changes the shape of the relationship and removes the obvious elbow point (interpretable as some sort of threshold) seen when the DTT is set at 5°C.

**Discussion**

Davies & Ratcliffe (1994) suggest that ADH values would be expected to be approximately similar for two congeneric species of similar size, such as *C. vicina* and *C. vomitoria*. The current study found that only the ADH to reach the 3rd

**Table 3.** Scheffe’s multiple comparison significance table between total ADH for differing treatments of 5-day cold episodes at various temperatures during the late 2nd larval stage for *Calliphora vicina* (top right) and *Calliphora vomitoria* (bottom left). Bold indicates significant at the 5% level.

<table>
<thead>
<tr>
<th></th>
<th>1°C</th>
<th>3°C</th>
<th>5°C</th>
<th>10°C</th>
<th>20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1°C</td>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3°C</td>
<td>&lt;0.001</td>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.903</td>
</tr>
<tr>
<td>5°C</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
<td>0.829</td>
<td>0.996</td>
</tr>
<tr>
<td>10°C</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.996</td>
<td></td>
<td>0.440</td>
</tr>
<tr>
<td>20°C</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.999</td>
<td>0.983</td>
<td></td>
</tr>
</tbody>
</table>

The estimation of the developmental threshold temperature DTT is normally calculated by extrapolating back the straight line plot of daily developmental rate against temperature to the point where it reaches zero (Williams & Richardson, 1984; Wall et al., 1992; Grassberger & Reiter, 2002). This method can be traced back at least to Wiggleworth (1965), who points out (p. 617) that at low temperatures the development velocity curve becomes non-linear and curves less steeply ‘so that the “development zero” is not in fact the true threshold of development; the temperature at which development ceases lies appreciably lower’. This is exactly as seen in Fig 5 where development either continues during the cold period but at a lower rate, or stops completely during the cold period and has some negative effect after the larvae are returned to 20°C: either way there is an increase in the total ADH for individuals subjected to low temperature episodes.

It could be postulated that the increase in development after the cold interval might be due to a period of diapause. However, once in diapause, specific stimuli are needed for development to resume. Vinogradova & Zinovjeva (1972) studied the factors leading to induction of diapause in C. vicina. It appears that diapause is only fully expressed below 3.5°C as hatching of C. vicina eggs occurred at this temperature. They also found that adults emerged at 5°C in their experiment. The authors then went on to suggest that if the minimum threshold for C. vicina is taken as 2°C, after Vinogradova & Marchenko (1984; cited in Davies & Ratcliffe, 1994), assuming that C. vicina and C. vomitoria have equal ADH above the thresholds, that the lower threshold for C. vomitoria can be estimated as 6°C. These points are not supported by this study, based on material caught in SE England. In their recent paper on the effects of refrigeration on the development of Protaphormia terraenovae, Myskowski & Doums (2002) also showed that development during the 4°C cold period is not totally arrested as is normally assumed by forensic investigators.

The concept of ADH assumes that there is a fixed quantity of metabolic activity, controlled by time and temperature, that is necessary to complete development. The linear model assumes that the time/temperature relationship is measured in terms of ADH, where one ADH unit is equal to 1 \((1^\circ \times 1\ h)\) across all temperatures. Figure 5 shows that, irrespective of what temperature is taken as the DTT, total ADH increases as a result of 5-day low temperature cold episodes. This may be interpreted in either of two mechanistic ways. Either the value of the ADH unit decreases with lower temperatures or the unit remains a constant but development is slowed so that there is an overall increase in the total ADH required. Either way, if the standard ADH method is used to estimate PMI where there have been periods of low temperature then it is likely that the PMI will be underestimated.

Published ADH values for a number of blowfly species have been collated by Higley & Haskell (2001). Their values for C. vicina and C. vomitoria (derived from data from Kamal, 1958 and Greenberg, 1991) show considerable variability both within their table (9.1) and with the data presented here. This suggests that the use of generic ADH values is suspect and may well vary on a climatic, locality, population or even possibly egg batch basis.

Kamal (1958) and Greenberg (1991) took 6°C as the minimum development threshold temperature for both C. vicina and C. vomitoria. Using species obtained from NE England, Davies & Ratcliffe (1994), however, suggested that C. vicina’s minimum development threshold must be
this current study, which took place during the summer months, the adult flies experienced long days and therefore diapause is probably not the cause of increased developmental times for those insects experiencing 5°C and below in larval stage 1. This is supported by the work of Vaz Nunes & Saunders (1989) who discovered that for C. vicina it was in the post-feeding stage that temperature was critical for averting diapause. In this project a cold spell experienced in larval 3 is not significantly different to larval 2 or pupal stages and therefore diapause is unlikely to be the cause of lengthened development after a cold episode in larval 1.

Johl & Anderson (1996) conducted a series of experiments where Canadian C. vicina larvae were chilled at different lifestages for 24 h at 3°C. They discovered that for those larvae chilled in larval stages 1, 2 and 3, development to adult emergence was delayed by about 1 day compared to the control. Myskowiak & Doums (2002) showed highly variable effects of refrigeration on the development of P. terraenovae, depending on the stage when cooling occurred. Paradoxically, they indicated that development time decreased if refrigeration occurred in the first or third larval stage but it increased if the cold spell was in the second larval or pupal stages.

Previous comparisons of temperature effects of different species have noted differences in development at various temperatures and related this to species distribution. Davies & Ratcliffe (1994) compared C. vicina with C. alpina Ringdahl (a Northern Holarctic species that coexists with C. vicina in the uplands of Britain) and suggested that C. vicina was cold-adapted. On this basis, it is possible that the same species from different places may also have different thermal constant ADH values. Greenberg (1991) compared adult ADH values for Lucilia (Phaenicia) sericata (Meigen) from the American Midwest to Marchenko’s (1980) data for the same species in Russia. The flies from the Midwest reached adult in 7684 ADH compared with 8395 ADH for the Russian strain.

Our study assumed that the control temperature (20°C) was itself a favourable temperature for both blowfly species and that 20°C falls on the linear region of the developmental rate/temperature relationship, where the ADH unit has a value of 1. This is a reasonable assumption for these two species as 20°C falls into the climatic conditions to which they would be adapted since both are temperate species. Campbell et al. (1974) consider insects to be so well adapted to their particular environment that ‘exposure to inescapable temperature extremes is rare’. They too assume that field temperatures will lie within the linear part of the temperature/development rate curve.

These findings add to the concerns expressed by Myskowiak & Doums (2002) that care should be exercised when applying ADH estimates to the calculation of PMI. Where cold periods, either natural or artificial (refrigeration), are included in the experience of the developing fly larvae or pupae, it should not be assumed that no growth has occurred during these cold periods.

Higley & Haskell (2001) have pointed to the paucity of information on DTT values for forensically important fly species. The whole concept of DTT and its input to the ADH calculation needs an urgent review in the light of the findings presented here and those of Myskowiak & Doums (2002).

The use of ADH is extensively used in PMI estimations and frequently provides results that are of value in forensic investigations. Its use, however, can become misleading when there are prolonged periods of cold weather. In one murder investigation, BDT used the ADH approach to find a minimum time of death using blowfly pupae from soil adjacent to a skeletonized corpse discovered in February in southern England. The calculation, which involved quite long periods in November and January–February where the temperature was below 5°C, proved to be in error by about a month. In this case the ADH method underestimated the amount of development that had occurred and so lengthened the PMI. Based on the ADH calculations, death was minimally estimated to be in mid September of the previous year whereas the murdered woman was in fact still alive a month later in mid October.

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