A MODEL FOR THE AGING OF FLY LARVAE IN FORENSIC ENTOMOLOGY

H. WILLIAMS

Department of Zoology, University of Tasmania, Box 252C, G.P.O., Hobart, Tasmania 7001 (Australia)

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Summary

A method is described for determining the time of hatching of blowfly larvae on a corpse with respect to temperature. Temperature is known to have a profound effect on the rate of growth of fly larvae, and it is suggested that past assumptions that the growth of larvae found in a corpse has taken place at one approximated ambient temperature may have led to large overestimates of the development time of the larvae, and thus overestimates of time of death.

Larvae of Lucilia cuprina, Calliphora vicina (=erythrocephala), Calliphora stygia and Calliphora hilli were cultured under constant temperatures, and their growth rates modeled with a logistic growth curve.

Two programmes have been developed, one to compute the parameters for the logistic equations, the other is used to estimate the time elapsed from the time at which a sample is removed from a body to the estimated hatching time of the larvae comprising the sample, with temperatures and species as variables.

Key words: Model for aging; Blowfly larvae; Hatching; Temperature

Introduction

This paper discusses a method for determining the time of egg hatching for some calliphorid fly larvae, from the weight of larvae taken at the time a corpse is found. Use of this method requires some knowledge of temperatures that the larvae are likely to have encountered previous to sampling.

As calliphorid larvae are usually the earliest arrivals of the decomposer community colonising corpses, an estimate of the date at which their eggs are laid represents a minimum estimate of the time of death. Calliphorid larvae are also the most dominant component of this community [1,2] and thus, when present, should be in large enough numbers to be sampled easily. Under normal circumstances the eggs of flies will be present on a corpse quite soon after death (1-2 days), if the body is accessible to flies.

Previous methods for estimating the development time of fly larvae have assumed an approximated ambient temperature, covering the whole of the
growth period. This is inadequate and leads to substantial errors for the following reasons.

Firstly, ambient temperatures are not necessarily good estimates of the temperatures occurring within a corpse. This has been demonstrated [3] where temperatures up to 45–49°C were recorded within a sheep carcass when the ambient temperature was approximately 18°C. This phenomenon is caused by the metabolic heat generated by larvae feeding within the corpse and to a smaller extent, by bacterial decomposition (which in turn is accelerated by the activity of larvae). Ambient temperatures can only be assumed therefore, when the level of larval infestation is very low and feeding clusters of larvae are composed of a few individuals.

The second reason is related to the effect of temperature on the growth rates of larvae. Growth rates change quite dramatically as the larvae progress through the various developmental stages, and the effects of temperature at different times during larval development will be equally dramatic. Thus, whereas one estimated temperature may be correct for the earlier stages of development when feeding clusters are exposed on the surface of the corpse, it may not be correct at the beginning of the third instar when the period of most rapid growth occurs [4]. As it is probable that the temperature at this later time will be significantly higher than ambient temperature for the reasons noted above, then the growth rate will also be higher and development time reduced more than expected. An estimate of growth rate using ambient temperatures, or one temperature covering the whole of the development period, will therefore grossly overestimate development time and consequently the estimate of time of death. For example, from Waterhouse's data an estimate of the time elapsed from hatching to sampling for L. cuprina larvae based on ambient temperature would be 192 h when in fact the time elapsed is 72 h.

The model developed here, used data for Calliphora vicina (=erythrocephala), C. hilli, C. stygia and Lucilia cuprina. However, the methods are applicable to any species whose larval growth can be satisfactorily modelled using the logistic growth equation. While this method will estimate time of hatching, further estimates are required to determine the length of the egg stage. These can be derived by the methods described in [7].

The model

For all species studied here, regression using a temperature dependent logistic curve fitted larval growth data well (Williams, H. and Richardson, A.M.M., unpublished). As growth can be described accurately by this method, it follows that given:

1. a temperature (upon which the growth rate and thus the parameters of a logistic growth curve are dependent);
(2) the time period over which this temperature affected larval growth; and

(3) the weight of larvae at the end of the time period stipulated in (2), an accurate estimate of the size of the larvae at the beginning of that time period can be extrapolated.

The logistic equation for growth at any constant temperature is:

\[ W_t = \frac{a/b}{1 + e^{(c-at)}} \]  \hspace{1cm} (1)

where \( W_t \) = weight at time \( t \); \( a, b, \) and \( c \) are constants; and \( e \) = base of natural logarithms.

As the regressions are based on constant temperatures the terms \( a, b \) and \( c \) are dependent on the temperature under which growth occurred. The parameters \( a, b \) and \( c \) are not linearly dependent on temperature and so estimates of growth occurring under fluctuating temperatures are made by extrapolation from the logistic parameters determined by regression at a temperature closest to the required temperature.

Because of this procedure, the term \( t \) in eqn. (1) has no real meaning as it refers to the time at which \( W_t \) is attained under constant temperatures. However, as weight is a known variable, eqn. (1) can be solved to find \( t \) and thus calculate the weight of larave at the beginning of a period of growth at temperature \( y^\circ C \) of duration \( x \) hours, by solving:

\[ t_{wy} = \frac{c_y - \left[ \ln \left( 1 + \frac{a_y/b_y}{W_s} \right) \right]}{a_y} \]  \hspace{1cm} (2)

where \( t_{wy} \) = time at which weight \( W_s \) is attained under constant temperature \( y \); \( W_s \) = the sample weight; and \( a_y, b_y \) and \( c_y \) are logistic constants for temperature \( y \).

The weight of larvae at the beginning of the time period can now be determined by solving:

\[ W(t-x) = \frac{a_y/b_y}{1 + e^{[c_y - a_y \cdot (t_y - x)]}} \]  \hspace{1cm} (3)

where \( x \) = length of the time period.

Disregarding \( t \) but summing the term \( x \) for each estimate of weight at the beginning of a growth period \( W(t-x) \) under temperature \( y \) and ending with
an estimate of weight $W_0$, the time elapsed since the larvae hatched (i.e. when egg weight is approached), can be calculated:

$$t_e = \frac{W_n}{\sum_{W_o} x}$$  \hspace{1cm} (4)

where $t_e =$ time elapsed; $W_n =$ weight of larvae at time of sampling; and $W_o =$ egg weight.

This argument forms the basis of the model for which a flow chart is given in Fig. 1.

Working from the known weight and species composition of a sample taken from a corpse, and with information on the likely mean temperatures encountered by the larvae over previous time periods, the model works through the expected temperatures and selects the most appropriate logistic parameters for each time period. This is repeated until the egg weight is approached. The real time elapsed is then output along with a correction for growth under fluctuating temperatures. This is included as the growth rate of *Lucilia illustris* has been found to be suppressed by 10% as compared with larvae grown under constant temperature condition [5], and it is assumed that this suppression of growth rate is shared with other species.

Logistic parameters for each species were determined over a range of temperatures (10–45°C in 5°C steps) by the method described in the next section.

**Using the model**

1. *Determining intrinsic values for the model*

The intrinsic values for this model are: the logistic growth parameters $a$, $b$ and $c$, the initial (i.e. egg) weights and the maximum weights attained (as larvae) for each species.

All weights used here are dry weights. Samples were dried for 24 h in a vacuum oven at 60°C.

The first requirement for acquiring data for the model is to determine the species of flies most often found in carrion and corpses in the locality. Cultures of these flies should then be used to supply adult flies for oviposition stocks.

Constant temperature facilities are needed to give temperatures within the thermal tolerance range of the larvae (this will normally be 10–40°C but may extend up to 45°C). The temperatures used in this study were 10–45°C in 5°C steps.

Egg clusters from the cultured stocks (or larvae in the case of ovoviviparous species) should be placed on a liver substrate at the required constant temperature. Some eggs should be retained and dried for determination of mean egg weights.
READ: Egg and maximum weight for all species.

READ: Logistic constants for thermal tolerance ranges.

READ: Species name, sample weight, and time of sampling.

READ: Expected temperature for previous 12h periods.

Is temperature within tolerance range?

F: No growth.

Time counter +12h.

T:

COMPUTE: Expected weight 12h ago.

Is expected weight less than egg weight?

F

T: a

When was egg weight reached in last 12h period?

OUTPUT: Hatching time.

COMPUTE: Hanski's correction i.e. add 10% to estimated time.

OUTPUT: Hanski's correction.

STOP

Fig. 1. Flow chart of the model used for estimating time of hatching.
Samples numbering approximately 20–30 of the ensuing larvae should be taken at intervals of 48 h for those developed at 10°C, 24 h for 15, 20, 25 and 30°C, and 12 h for 35, 40 and 45°C.

These samples are then dried as described before and weighed individually on a microbalance. From this data a mean weight, and the time at which it was attained should be recorded. Sampling should cease when the mean weights of subsequent samples start to decline. This occurs when the larvae leave the substrate and prepare for pupation.

This information can then be used to determine the logistic parameters for the species’ growth at a given temperature. Further information on the use of this data in the programme is outlined in the instructions for use of the programme, which is available on request from the author.

2. Procedure for use

The model requires (as input data) the following information:

(1) the mean weight of larvae removed from a corpse;
(2) the sample to be of one identified species with growth curves described for it;
(3) the time at which the sample was taken; and
(4) a series of temperatures which are expected to have affected the growth of the larvae in previous time periods (of 12 h lengths).

Samples removed from a corpse should consist of about 30–50 individuals, to ensure enough for both identification and the derivation of mean weights. It is preferable that samples be taken from the head if possible, as this is usually the area colonised earliest and is thus a more accurate indicator of time of death. The sample should be placed directly into vermiculite or sand, and no food included as it is the weight of the sample at the time it was taken that is needed. The larvae should not be preserved, as the various preserving agents will affect the dry weight. The sample should then be dried and weighed as described before and the mean weight calculated.

Temperatures which are likely to have affected larval growth are left to the discretion of the entomologists using this model. They should bear in mind that the situation of the body (i.e. in direct sunlight or shade), the level of infestation and the ambient temperature for previous night and day periods will all affect the temperatures likely to have occurred inside the corpse. At the present time much of this data will be arrived at by informed guess work. These problems have been recognised elsewhere [7] and it is hoped that data collected in the future will allow for the development of methods for interpolating temperatures.
Discussion

The logistic growth curve has been fitted with a significant level of agreement to many species of calliphorid flies, notably *L. cuprina*, *C. vicina*, *C. hilli* ([Williams, H. and Richardson, A.M.M., unpublished]); *L. illustris* [5]; *L. cuprina*, *C. augur*, *C. nociva*, *C. stygia*, *Chrysomya varipes*, *Ch. rufifacies*, *Ch. megacephala* and *Parasarcophaga crassipalpis* [4]; *L. sericata*, *Ch. marginslis*, *Ch. albiceps*, and *Ch. chloropyga* [6]. It is therefore reasonable to assume that it should be adequate for other calliphorid fly larvae.

Although temperature is possibly the most important variable determining the rate of growth, it has been demonstrated that competition by overcrowding within a carcass will suppress the size of larvae while maintaining the length of time they will spend in the feeding stage [6]. Thus, in cases where overcrowding is observed, it is likely that the model will give an underestimate of development time due to the smaller size of the larvae. There appears to be little work in the literature on this phenomenon apart from that of [6] but it is hoped that compensations for this factor may be determined in the future.

The largest error possible in the model is the lack of data on carcass temperatures in relation to larval infestation in the literature, the only information available is presented in [3]. If the temperatures are known accurately the estimate of hatching time should be accordingly accurate. Sufficient accuracy of temperature estimates can only be obtained by collecting information relating to corpse temperatures, and the conditions surrounding these temperatures, e.g. level of infestation, species composition, aspect of the body etc. from actual cases. When this information is known, the model described here should be reliable, since it is a deterministic model, i.e. with a known outcome when the variables are defined. As temperature is the only variable recognised by the model, the accuracy of predictions are reliant on the accuracy with which that variable is determined.

In cases where infestation is very low or the corpse is small, such as a baby, it is safe to assume ambient temperatures [7], but they should be mean values for day and night periods.

One further factor limiting the use of the model is the condition that the larvae making up the sample are actually from eggs laid soon after the time of death. The period from death to oviposition can be variable [8]; in some cases the corpse may be isolated from flies for an unknown period, or weather conditions may protect the corpse from flies (e.g. by maintaining temperatures below the flight threshold temperatures). It is also possible for the initial cohort of larvae to develop on the corpse, to be followed by subsequent cohorts of larvae as long as the corpse remains a suitable habitat for the development of larvae.

These problems cannot be dealt with by the model and it is up to the forensic entomologist conducting the investigation to adjust for them.
So far as the model has been used by the author, the estimates generated have either agreed well with determinations made by standard medical forensic means or supplied a definite minimum time since death where no medical estimate was certain (Appendix 1). The two programmes (one for estimating time of hatching and the other for computing the logistic parameters) and data relating to the species studied here are available on request from the author. They are written in FORTRAN IV and are thus compatible with most computer systems.

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Appendix 1

*Case 1*

Last seen; 19/11/82 8.30 p.m.

Body found; 29/11/82 10.15 p.m.

The body was found partially submerged in running water. Larvae removed from the head were identified as *Calliphora hilli* and *Calliphora stygia*.

The mean dry weight of larvae were 15.11 mg and 4.52 mg for *C. hilli* and *C. stygia*, respectively.

As the body was nearly completely submerged it was assumed that the water temperature (15°C) would have had the greatest effect on body temperature and thus the temperature affecting the development of the larvae.

Using a constant 15°C an estimate of hatching time of 48–72 h and 96 h (before sampling) was found for *C. hilli* and *C. stygia*, respectively.

It was concluded that the body was accessible to flies at least by 25/11/82. The pathologist's report concluded that death was due to drowning in fresh water and an estimated time of death was 'several days at least' based on the state of decomposition of the body.

*Case 2*

Last seen; Unknown.

Body found; 29/8/82.

The body was found in the home of the deceased. A sample of larvae was removed from the mouth and identified as being composed entirely of *Calliphora vicina*.

The mean dry weight of the larvac was 24.71 mg, and a temperature
regime for development was assumed to be modulating between 15°C and 20°C (night and day).

This data gave an estimate of 8 days from hatching and allowing 12 h for hatching, approximately 8.5 days since the body was accessible to flies.

The pathologist's report gave an estimated time of death of between 7 and 8 days based on the degree of decomposition and the absence of rigor mortis.

References