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Development of immature blowflies and their application to forensic science

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A thesis submitted by Donnah Marie Day for the degree of Masters of Science - Research at the University of Wollongong, NSW.

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I, Donnah Marie Day, declare that the work recorded in this thesis is entirely my own effort, except where otherwise acknowledged. I also certify that this work is original and has not been previously submitted in any other course of study at this or any other institution.

Signature Date

Student number

All nature is lovely and worthy of our reverent study.

Anonymous

ABSTRACT

Data on the development of immature blowflies and other carrion-breeding flies can assist with determination of the post-mortem interval and thus be used as a tool to help solve crime. The main focus of this research was to develop reliable data for growth at constant temperatures in larvae of *Calliphora augur*. Constant temperatures were chosen because fluctuating regimes can be location specific and may therefore have limited application. A number of other blowfly species were also studied, but only *C. augur* and *Lucilia cuprina* were used in planned experiments. Since *C. augur* is ovoviviparous, and therefore has a small clutch size, the fecund egg-laying species *Lucilia cuprina* was also cultured and used as a model for pilot experiments, in feasibility studies and to explore the broader applicability of results from trials with *C. augur*.

Some of the current ideas behind estimation of post-mortem interval using blowfly larvae have been expanded upon and prediction intervals for larvae of *C. augur* are presented. In forensic entomology, plots of this type usually present the variables differently than the strict mathematical method, where a known predictor (x) is used to estimate an unknown value of interest (y). In forensic entomology, these axes are often reversed. In my work, I have adopted a more classical mathematical method and present a way of estimating time as related to larval age (y) from the (known) somatic measurement of body length (x). Whilst this has been the main core of my work, some important practical difficulties of working in this field have also been given attention.

A way to salvage some damaged specimens has been discovered; measurement of body width at the junction of the 5^{th} and 6^{th} abdominal segments can be used as an alternative measurement to body length, and I present a means to convert from one measurement to the other. A paper on this topic has been published in *Forensic Science International*.

I have also explored the effect of freezing and thawing developmental media on larval growth in *Calliphora augur* and *Lucilia cuprina*, and it appears that there is no significant difference. The effect of different tissues from sheep on larval growth was also examined in the above two species, with growth on sheep's liver being slower than growth on sheep's brain or sheep's meat. A paper on this topic has been accepted by the *Journal of Forensic Sciences*.

The effect of preservative solutions on different larval stages of *C. augur*, and on the third instar larvae of *C.augur* and *L. cuprina*, was also examined. It was found that the larvae of each species reacted differently to the preservatives, as did the different instars within a

species. Ten per cent formalin and Kahle's solution effected the least change in larval body length, but when larvae were placed into preservatives alive only 10% formalin had no deleterious effect on both species. However, since 10% formalin can affect the analysis of larval DNA, it is not recommended. In fact, it appears that choosing an optimum preservative may be difficult until more work is done in this area.

While work in forensic entomology is far from straightforward, it is hoped that the decidedly practical nature of my studies will serve to equip forensic entomologists with more tools to help solve crime.

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ABBREVIATIONS

PMI	post-mortem interval
DNA	deoxyribonucleic acid
sp.	species
C.	*Calliphora*
Ch.	*Chrysomya*
L.	*Lucilia*
%	percent
+	plus
±	plus or minus
pers comm.	personal communication
e.g.	for example
i.e.	that is
c.f.	compare with
vs.	versus
am	morning
pm	afternoon/evening
mm	millimetres
cm	centimetres
km	kilometres
mcg	microgram
mg	milligram
g	gram
mL	millilitre
h	hour
R-sq	R-square
R-sq adj	R-square adjusted
ANOVA	analysis of variance
d.f.	degrees of freedom
std dev.	standard deviation
p=	probability
P	probability
n=	number
°C	degrees Celsius
EtOH	ethanol
CO_2	carbon dioxide
E	east
S	south

I sincerely hope you never have need of these findings.