

## The development of a predictive model for spring emergence of *Lucilia cuprina*.

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### Summary

*The aims of this study were to examine the overwintering behaviour of L. cuprina and to use these results to develop a predictive model for spring emergence. In the colder areas of its range, this species is known to remain dormant in the soil in the post-feeding larval stage until the following spring. This study has shown that the mechanism of dormancy is a facultative diapause and this overwintering strategy can be flexible. Lucilia cuprina has the ability to keep developing into winter if temperatures are warm enough and spring emergence can vary from year to year, depending on temperatures experienced in the colder months. Although this study was unable to present a final model to predict emergence in the field it was able to determine the deficiencies in available data at present and test some of the models available in the literature.*

### Keywords

Blowfly, *Lucilia cuprina*, overwintering, facultative diapause, development, predictive model.

### Introduction

Knowledge of the seasonal variability of blowfly species is important in developing management strategies against blowfly strike. If graziers can be pre-warned of impending spring emergence they can implement preventative management practices and retard the critical initial population build up at the start of the season and reduce future blowfly populations (Barton, 1982; Anderson *et al.*, 1984; Anderson *et al.*, 1990). Many species of blowflies are known to overwinter, with the majority entering some form of diapause. In the colder southern part of its range in Australia, once temperatures drop in autumn, *L. cuprina* is known to remain dormant in the soil in the post-feeding larval stage until the following spring (Norris 1959; Foster and Helman 1979; Dallwitz and Wardhaugh 1984). The mechanism for overwintering of *L. cuprina* has not been described. Dallwitz and Wardhaugh (1984) suggested two main influences on overwintering of this species; i) the temperature experienced by the post-feeding larvae, and ii) the photoperiod experienced by their mothers. The aim of this study was to examine the overwintering behaviour of *L. cuprina*. These results were used to develop a predictive model for spring emergence based on development as a function of soil temperature.

### Methods

#### General

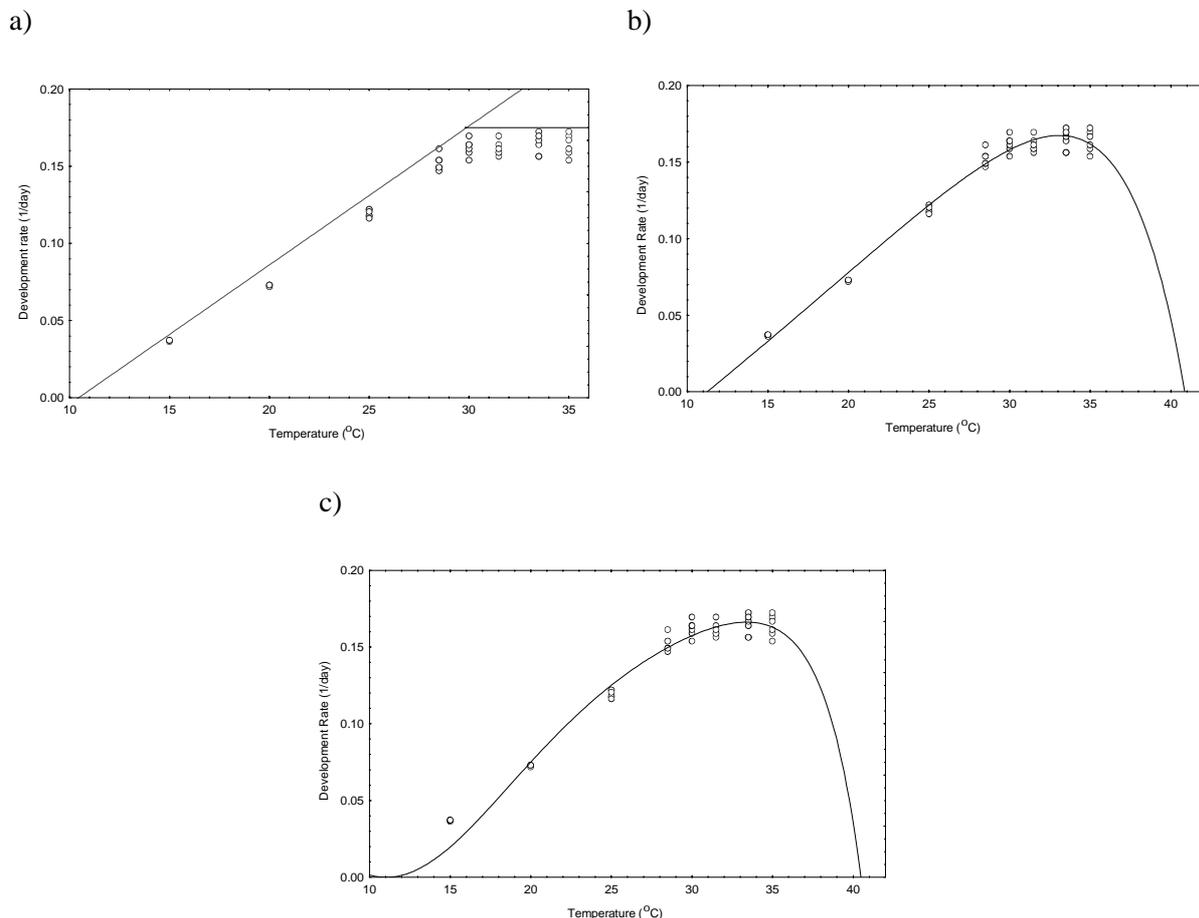
Trials were conducted over three consecutive winters at the Fowlers Gap Research Station in far-western New South Wales. Post-feeding larvae used in these trials were bred from either the laboratory strain (McLeod, 1997), or from wild females trapped one day prior to egging (Winter 1 only). The larvae of the laboratory strain were kept under the conditions of 25 - 30°C and LD 12:12, while the larvae of the field strain were kept under natural temperature and light regimes. The experimental pots were made of PVC pipe (diameter 11.0 cm, depth 15.0 cm) with a fly wire base and a removable flywire lid (McKenzie, 1990).

The control pots were kept in the laboratory at 25°C and the field pots were placed in the ground so that the top of the pot was 3.0 cm above the surface level of the surrounding soil, and 25.0 cm apart. Samples of fifty larvae per pot were used. Allocation of treatments and species/strain combinations followed a randomised row design. At least five replicates were used for each combination. The pots

were checked daily for fly emergence. Carbon dioxide gas was used to anaesthetise the newly emerged flies for counting and sexing.

### Models

This study compared the fit of a linear degree-day model and two nonlinear functions; the matched-asymptotic model derived by Hilbert and Logan (1983) (labelled “Equation 6”) and the second modified Logan model from Lactin *et al.* (1995), to the development results for *L. cuprina* to determine the best model (see McLeod, 1997 for detailed description). The development times for *L. cuprina* pupae were taken from the constant temperature studies of Dallwitz (1984), with the rate of pupal development and temperature close to linear between 15 and 30 °C (Figure 1). The base temperature was calculated to be 11.03 °C ( $y = 0.009x - 0.095$ ,  $r^2 = 0.99$ ). There is no accurate data available for the development times for *L. cuprina* wandering larvae in the literature, so the *L. sericata* data of Wall *et al.* (1992) was used. The relationship between the rate of pupal development and temperature for *L. sericata* is similar to that for *L. cuprina* (McLeod, 1997). For the wandering larvae of *L. sericata*, the base temperature was calculated to be 10.65°C ( $y = 0.026x - 0.277$ ,  $r^2 = 0.92$ ). All three models gave reasonable good fits to the available data ( $r^2$  between 0.92 and 0.94 for post-feeding larvae development stage and between 0.96-0.99 for pupal development data - McLeod, 1997). An *a priori* decision could not be made on which model was best for this study so all three models were solved.



**Figure 1. a) Linear degree-day model,  $r^2 = 0.99$ ; b) Modified Logan model,  $r^2 = 0.98$ ; c) Matched-asymptotic model (Equation 6),  $r^2 = 0.96$  fitted to *L. cuprina* pupal development data from Dallwitz (1984).**

### Development Rates

Expected times for emergence from each trial were calculated using hourly soil temperature measured just below the soil surface. When calculating the development rates, the wandering larval development was taken from the time the larvae left the food until pupariation. Pupal development was taken as the time from the start of pupariation until emergence of the adult fly. It was assumed that post-overwintering development responded to the physical factors (primarily temperature) in a

similar manner as non-overwintering development (Tauber and Tauber, 1976), and that no diapause occurred during the pupal stage. Simulations of developmental rate were solved using Monte Carlo simulation methods to incorporate variance in the model's parameters. Used in this way Monte Carlo methods can generate a mean and confidence interval for the predicted developmental times (Manly 1991; Manly, 1992). For each iteration of the simulation, new parameter estimates were taken as a random draw from a normal distribution of one standard error around the mean of each variable. These predicted times of emergence could then be compared to the observed data.

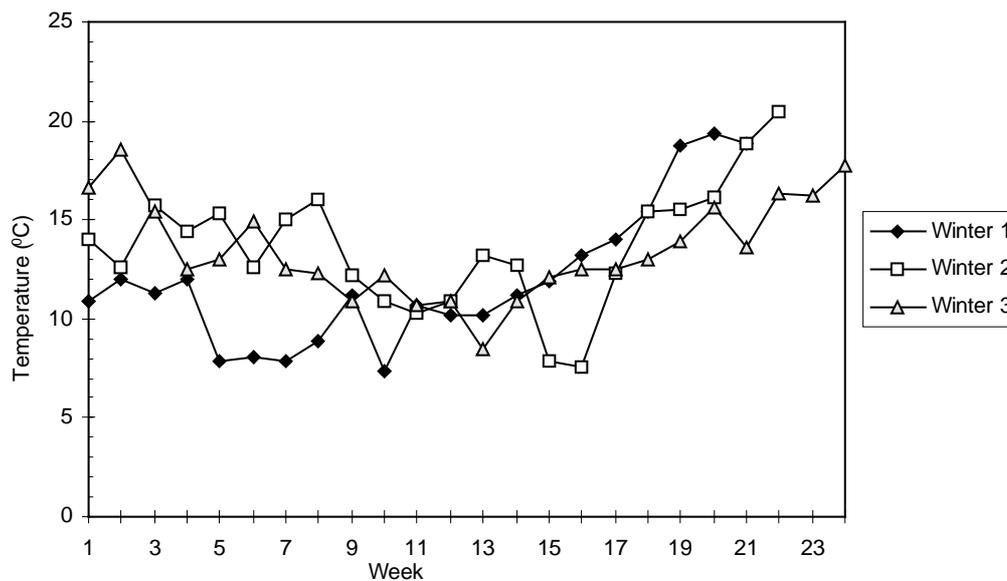
### ***Termination of Diapause***

Since the timing of emergence was known, developmental units were calculated, using the linear degree day model, to work backwards so as to determine the exact time that the overwintering larvae terminated their diapause and recommenced their normal development.

## **Results**

### ***Temperature Conditions***

The temperature conditions experienced varied over the three winters of this study. The first winter experienced its coldest temperatures at the start of the season, whereas the second and third winters remained relatively mild until the later in the season (Figure 2). Minimum average weekly soil temperatures at 5 cm depth for each winter trials were 7.4<sup>0</sup>C for winter 1, 7.5<sup>0</sup>C for winter 2 and 8.4<sup>0</sup>C for winter 3. Maximum average weekly soil temperatures at 5cm depth were 36.5<sup>0</sup>C for winter 1, 42.8<sup>0</sup>C for winter 2 and 36.5<sup>0</sup>C for winter 3.



**Figure 2. Average weekly minimum temperatures recorded at soil depth 5cm for the three winter trials (Week 1 commencement of each trial - around late April early May).**

### ***Field Emergence***

Emergence from the control pots averaged 91.6%, taking an average of 8.1 days until first emergence, and occurred over 2-3 days (average for total emergence was 8.6 days). Emergence from the field pots varied between 21% to 75%. An example of the results for daily emergence from the field pots in the three trials are given in Figure 3. The first trial W1 was the only one where the majority of larvae remained dormant for the duration of the winter months. In both the second (W2) and third (W3) trials, the majority of larvae showed delayed pupariation but emerged throughout winter. Only a small number remained in the soil for the duration of winter to emerge in September.

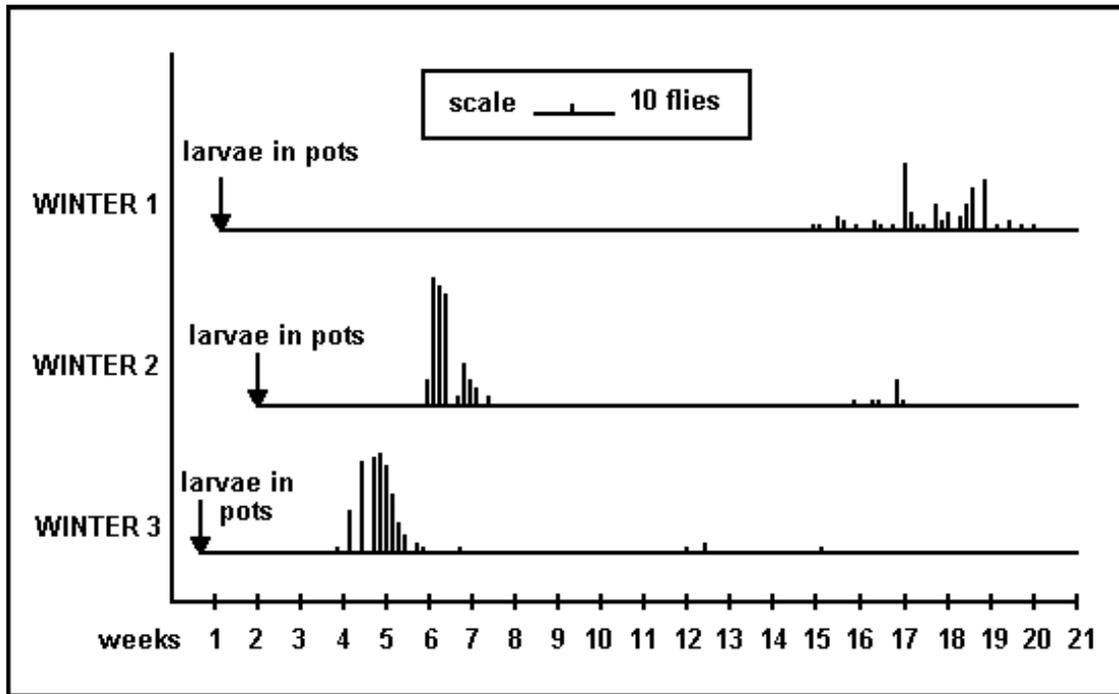


Figure 3. Daily emergence of *Lucilia cuprina* during the three winter trials.

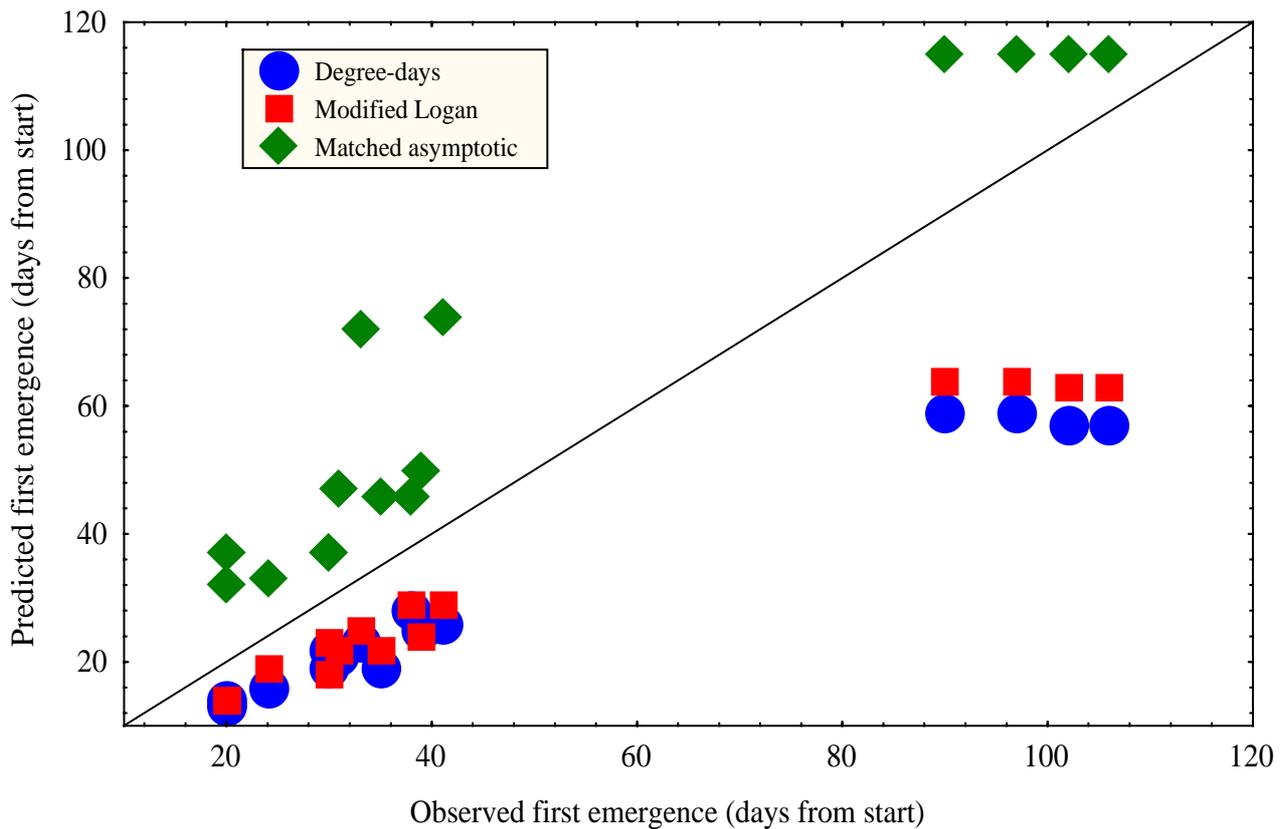


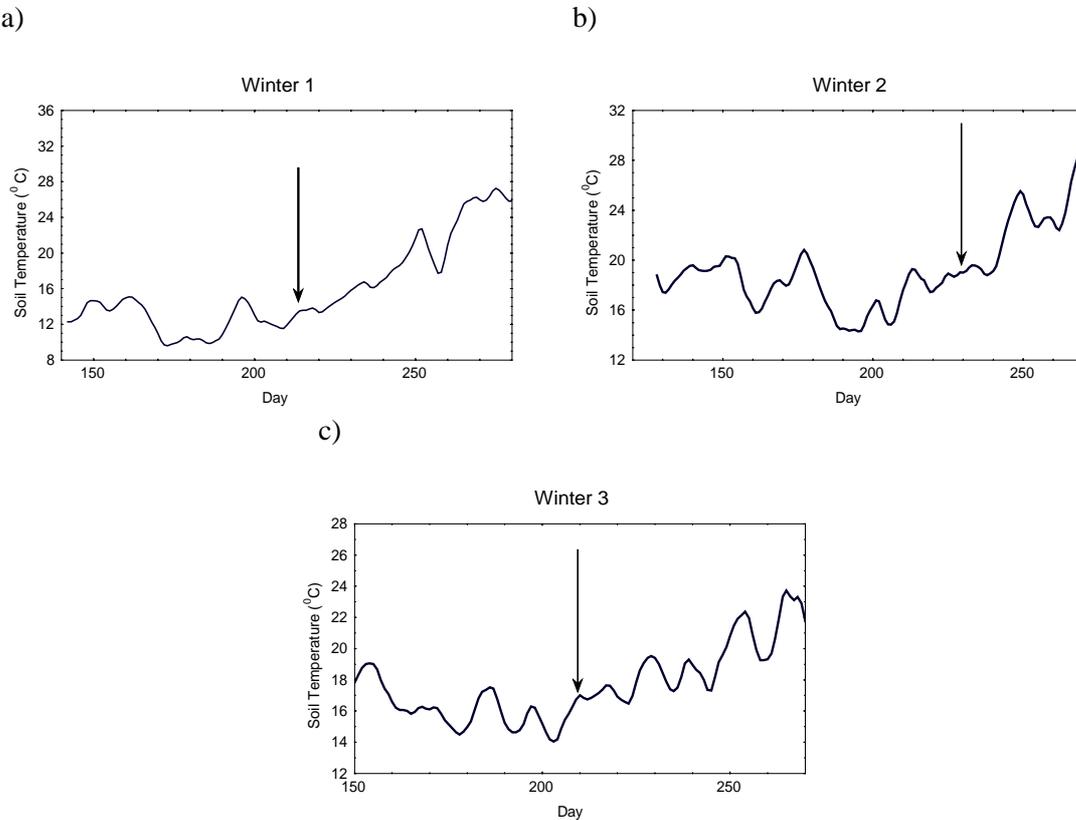
Figure 4. The observed emergence plotted against the predicted emergence from the three tested models ('Degree Days', 'Modified Logan' and 'Equation 6').

**Development Rates**

The observed field emergence is plotted against the predicted emergence from the tested models ('Degree Days', 'Modified Logan' and 'Equation 6') in Figure 4. The points to the far right of this plot represent the data from Winter 1, whilst the other points to the left are from Winters 2 and 3.

### Termination of Diapause

The calculated timing of the recommencement of normal development in the overwintering individuals in each winter is indicated in Figures 5a, b and c. In Winter 1 development recommenced with the soil temperature at 5 cm depth around 13°C. In Winters 2 and 3, the soil temperature was higher (between 17-18°C). In all three winters development appeared to recommence after a rise in soil temperatures. However it is not clear why those particular rises were any different from other instances where temperature had undergone a similar increase.



**Figure 5. Average soil temperature at 5 cm depth with a 6 point moving average to smooth curves for a) Winter 1, b) Winter 2 and c) Winter 3. Arrow indicates predicted termination of diapause.**

### Discussion

The temperatures experienced over the three winters of this study varied and the resulting emergence patterns are probably a reflection of these varying conditions. The first winter experienced its coldest temperatures at the start of the season and the majority of flies in this winter remained dormant for the duration of the winter months, with only a small number emerging in July (close to the dates predicted by the temperature development rate models). In the second and third winters temperature conditions remained relatively mild until the second half of the season. The majority of flies in these two winters emerged in the earlier months of winter, close to the dates predicted by the temperature development rate models and only a small number showed a delay until late winter/early spring.

From the results of their overwintering experiments on *L. cuprina* in the Canberra region, Dallwitz and Wardhaugh (1984) concluded that late March/April was a transition period with some individuals pupating in this time but an increasing number ceasing development until spring. After mid-April no pupation occurred and emergence was in spring. Spring emergence was synchronous for all individuals. Larvae collected from the summer until late-March had completed pupation by an average of 8.2 days, whereas larvae collected from late-March onwards took several weeks to pupate (average 15.3 days). These delays in pupation occurred more frequently in larvae collected after late-March leading the authors to speculate that the seasonal pattern of dormancy is developed in an earlier life stage and that the maternal photoperiod may be an important influence. Also that

conditions (temperature) experienced by the wandering larvae themselves were also important in determining the overwintering state.

In the winter 1 trial, larvae from 'laboratory-bred' flies (12:12 light regime) and 'field-bred' flies (natural light regime) were used in an attempt to test Dallwitz and Wardhaugh's (1984) hypothesis that maternal photoperiod may influence the overwintering behaviour of its offspring. No difference in the emergence times was found between these two types. One explanation for this result could be due to the insufficient time the 'laboratory bred' flies were kept under laboratory conditions. This trial was at the start of the project and the 'laboratory bred' females egged for the trial were only the second generation kept at the 12:12 light regime. In most insect species that have been studied, a number of inductive cycles are required to produce any measurable effect on photoperiodic responses (Saunders 1982), therefore these second generation females may not have been kept for a long enough period at the 12:12 light regime. Without further replications of this comparison of 'laboratory bred' vs 'field-bred' with the 'laboratory bred' flies held at 12:12 for a longer period, any difference in the overwintering behaviour of the offspring can not be determined from this study, and therefore the hypothesis of Dallwitz and Wardhaugh (1984) remains untested.

Because of the inability of this study to determine the effect, if any, of maternal photoperiod on overwintering behaviour and the use of 'laboratory-bred' flies for the majority of the trials, the results obtained may not be directly relevant to the field situation. But by controlling for any photoperiod effect, this study was able to test different predictive models based on temperature dependent development in a field situation. The emergence results from all three models tested were reasonably close in predicting the first wave of emergence in all trials but not exact. The predictions of the modified Logan equation were similar to those of the linear thermal summation model on most occasions, owing to the fact that the Logan model is linear in the range of temperatures experienced in this study. It is speculated that development at these lower temperatures is nonlinear but without more detailed development data, this relationship can not be determined.

There were several sources of inaccuracies when calculating these models, mainly due to the lack of published development data for *L. cuprina*, and these inaccuracies would need to be improved before any model could be fully assessed. More detailed work on the development rates for the pupal stage of *L. cuprina* under low temperatures is required if accurate models are to be developed. The experimental methodology of these trials would also have caused some inconsistencies in the development rate calculations, particularly the collection of wandering larvae to be placed in the field pots. Wandering larvae were only collected once before dawn, therefore there could have been a difference of several hours in the time they had spent in the wandering stage at the constant laboratory temperature.

Although the first wave of emergence was closely predicted by temperature dependent models, the second wave was not. These second waves of emergence represent the overwintering proportion of the population, where the larvae remain dormant for some period before recommencing their development. The majority of individuals overwintered in winter 1 and only a small number overwintered in winter 2 and winter 3 leading to the speculation that temperature conditions experienced by the wandering larvae play an important role in the 'decision' to overwinter. The mechanism for this dormancy is still unclear, as is the trigger for the termination of the dormancy. From the results of this study it is suggested that *Lucilia cuprina* overwinter in a facultative diapause. An interruption in their development is anticipated by the larvae, programmed by maternal photoperiod or some other condition experienced at an earlier stage of development, but the temperature they experience is also an important influence on deciding their overwintering behaviour. From preliminary analysis it appears that an increase in temperature forms part of the termination trigger, but more research is required to clarify this aspect as well as other conditions that may be involved.

Diapause studies of other species of blowflies outside of Australia have never reported the occurrence of a facultative diapause, although most do not specify an obligate diapause either. Other than *L. cuprina* there have been no other overwintering studies conducted on blowflies in Australia, however other species of insects have been studied. One of the best documented cases of facultative

diapause in Australia is that exhibited by the Australian plague locust, *Chortoicetes terminifera*. (Wardhaugh 1986). The ability for the Australian plague locust to survive over such a wide area is enhanced by its overwintering choices. Although the overwintering behaviour of *L. cuprina* does not seem to be as complex as that for the Australian plague locust, its ability to survive over the wide range of conditions experienced throughout Australia must also be enhanced by its ability to overwinter when conditions are too cold, but continue developing if conditions allow.

## Conclusion

This study investigated the overwintering behaviour of *L. cuprina*. Temperature is an important seasonal factor limiting this species. In the colder southern part of its range, once temperatures drop in autumn, *L. cuprina* is known to remain dormant in the soil in the post-feeding larval stage until the following spring. But this study has shown that this overwintering strategy is flexible and that *L. cuprina* has the ability to keep developing into winter if temperatures are warm enough and spring emergence can vary from year to year. This can have major implications on the effectiveness of a control program.

The highly seasonal nature of *L. cuprina* should make the development and incorporation of a predictive model for spring emergence of this species an important tool for use in an integrated management plan. Although this study was unable to present a final model to predict emergence in the field it was able to determine the deficiencies in available data at present and test some of the models available in the literature. The lack of development data for *L. cuprina*, especially at low temperatures is the main weakness in current published knowledge. More field studies are required to study the overwintering behaviour of *L. cuprina*. From this improved data, a general predictive model for *L. cuprina* could be developed. Non-linear models seem more appropriate as the development rate at the low temperatures, as experienced in winter, tends not to be linear for this species.

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