CANADIAN POLICE RESEARCH CENTRE

CENTRE CANADIEN DE RECHERCHES POLICIÈRES

## Technical Report

# TR-04-2005

# DEVELOPMENTAL RATE OF *PROTOPHORMIA TERRAENOVAE* (R-D) RAISED UNDER CONSTANT AND FLUCTUATING TEMPERATURES, FOR USE IN DETERMINING TIME SINCE DEATH IN NATURAL OUTDOOR CONDITIONS,IN THE EARLY POSTMORTEM INTERVAL.

**Prepared by**


**Colin A. Clarkson, B.Sc.**

**Niki R. Hobischak, B.Sc. (Hon.), M.P.M.**

**and Gail S. Anderson, M.P.M., Ph.D.**

**This report is a publication of the Canadian Police Research Centre.**
**For additional copies or further information contact:**


| | |
|---|---|
| Canadian Police Research Centre (CPRC)<br>Building M-55, 1200 Montreal Road<br>Ottawa, ON K1A 0R6<br>Telephone: (613) 990-8577<br>Fax: (613) 949-3056<br>**www.cprc.org** | Centre canadien de recherches policières (CCRP)<br>Édifice M-55, 1200, chemin de Montréal<br>Ottawa (Ontario) K1A 0R6<br>Téléphone : (613) 990-8577<br>Télécopieur : (613) 949-3056<br>**www.cprc.org** |

**TECHNICAL REPORT**
**CANADIAN POLICE RESEARCH CENTRE**

# DEVELOPMENTAL RATE OF *PROTOPHORMIA TERRAENOVAE* (R-D) RAISED UNDER CONSTANT AND FLUCTUATING TEMPERATURES, FOR USE IN DETERMINING TIME SINCE DEATH IN NATURAL OUTDOOR CONDITIONS,IN THE EARLY POSTMORTEM INTERVAL


*Colin A. Clarkson, B.Sc.*

*Niki R. Hobischak, B.Sc. (Hon.), M.P.M.*

*and*

*Gail S. Anderson, M.P.M., Ph.D.*

*Forensic Entomology Laboratory*

*Simon Fraser University*

*8888 University Dr,*

*Burnaby, B.C. V5A 1S6*

## EXECUTIVE SUMMARY

Forensic entomology is the study of insects associated with a dead body in order to determine time since death, and is a valuable tool in homicide investigations. To be of value, developmental data must be generated for a specific species of insect, at temperatures that are consistent with those found at crime scenes. This is the first of several small experiments to study the effects of different temperatures on the development of *Protophormia terraenovae*, a species of blow fly.

The objective of this study was to determine if *Protophormia terraenovae* developmental rates differ when they are raised under constant versus fluctuating temperatures. These data can now be used in death investigations in this region of British Columbia.

**DEVELOPMENTAL RATE OF *PROTOPHORMIA TERRAENOVAE* (ROBINEAU-DESVOIDY) RAISED UNDER CONSTANT AND FLUCTUATING TEMPERATURES**

Colin A. Clarkson, B.Sc., Niki R. Hobischak, B.Sc. (Hon.), M.P.M., and Gail S. Anderson, M.P.M., Ph.D., School of Criminology, Simon Fraser University, Burnaby, B.C.

## ABSTRACT

*Protophormia terraenovae* (Robineau-Desvoidy) (Diptera Calliphoridae) were raised from egg to adult outdoors under naturally fluctuating temperatures. Minimum times to reach the five developmental stages were noted (1st, 2nd, and 3rd instar, pupae, and adult). Once the adult stage was reached, the mean temperature during each of the stages was calculated, in addition to the overall mean temperature for the experiment. Next, *P. terraenovae* were raised from egg to adult within an incubator in the laboratory at a constant temperature of 20 °C. This is the same as the overall mean temperature recorded for the initial experiment done under fluctuating temperatures. Accumulated degree hours (ADH) were calculated for both experiments, at all five developmental stages, and were then compared between experiments. Differences in developmental rates for *P. terraenovae* raised under the two temperature regimes were observed for 1st, 2nd, and 3rd instar stages; however, there was no difference in the development of the pupae and adult stages.

## ABSTRACT (Non-Technical)

Accurate insect development data is essential when trying to determine time since death (post mortem interval). Any differences between development rates determined in the laboratory and those at an actual crime scene could potentially lead to an inaccurate time of death calculation.

Experiments were conducted to compare the difference in developmental rates of *Protophormia terraenovae* raised under naturally fluctuating temperatures and those raised at a constant temperature, for each of the five stages of development (1st, 2nd, and 3rd instar, pupae, and adult).

## INTRODUCTION

Blow flies (Diptera: Calliphoridae) are often used as forensic indicators of time since death (Marchenko 2001; Anderson 2000; Greenberg 1991). Accurate development rates of these blow flies are vital to determine time since death in homicide investigations. Most studies have determined development rates at various constant temperatures (Grassberger and Reiter 2002; Marchenko 2001; Anderson 2000; Greenberg and Tantawi 1993; Kamal 1958), but few experiments have been performed under the natural, fluctuating temperatures often found at crime scenes, particularly those found in British Columbia.

Literature suggests that for a variety of insect species including blow flies, rates of development are different when raised under fluctuating temperatures compared to a corresponding constant temperature (Ratte 1984). Therefore, any differences between development rates determined in the lab (constant temperatures) and those at an actual crime scene (fluctuating temperatures) could potentially lead to inaccurate time of death calculations. The objective of this study was to determine if the developmental rates of *Protophormia terraenovae* differed when they were raised under constant versus fluctuating temperatures.

## MATERIALS AND METHODS

Larvae of *P. terraenovae* were collected from pig carcasses in Maple Ridge, British Columbia, located in the Coastal Western Hemlock Biogeoclimatic Zone (Meidinger and Pojar 1991). Captured insects were used to establish a laboratory colony at Simon Fraser University. They were raised in wire mesh cages (45 cm x 45 cm x 45 cm), and supplied with water, cubed sugar, and milk powder *ad libidum*. When eggs were required, a small black 35 mm film container was partially filled with fresh beef liver, and placed inside the cage (Grassberger and Reiter 2002). The container was checked visually every 15 minutes for time of first oviposition. Following first oviposition, the container was left in the cage for two hours, to allow for a mass of eggs to be laid on the liver. The time when the liver was finally removed from the cage was recorded as the beginning of development (i.e. age of 0 hours was recorded for the eggs).

Each experiment consisted of 3 replicate 1-gallon (4.5L) glass jars, and 1 control jar. Approximately 4 cm of water-dampened sawdust was placed on the bottom of each jar. Folded paper towel was set on top of the sawdust, and approximately 250 g of raw beef liver was placed on the paper towel. Approximately 200 eggs were spread onto the beef liver in each of the 4 jars. All 800 eggs (200 per jar) came from one film container. The jar openings were then covered with paper towel and a layer of thin cloth mesh, and secured with elastic bands.

The jars were randomly assigned as Experimental Jar 1, 2, 3, or Control. The control jar was used to determine whether handling the larvae as they developed had any effect on development rates. None of the larvae in the control jars were handled; instead, they were inspected visually at

the same intervals as the experimental jars were checked. Therefore, observations for time to reach 2nd and 3rd instar could not be determined and were not recorded.

***Experiment 1***

Four jars were placed outdoors on an apartment balcony in New Westminster, British Columbia. They were protected from rain and direct sunlight by a roof, but they were still exposed to natural temperature fluctuations. A data logger (Smartbutton® data logger, ACR Systems Inc.) was placed along side the jars, which recorded the temperature at 30-minute intervals for the duration of the experiment. The experiment started in late May of 2003 and continued until late June of 2003.

Jars were usually inspected every 4 to 6 hours. Minimum times to reach 1st instar, 2nd instar, 3rd instar, pupae, and adult stages were established. The entire cohort in each jar was observed for evidence of egg hatch (1st instar stage reached), pupation, and adult emergence. Samples of 10 larvae were removed and examined under a light microscope at each examination time to determine when 2nd and 3rd instar stages were reached.

A jar was considered to have reached a particular stage of development when approximately 10% of the jar's population reached that stage. The figure of 10% had to be estimated visually for egg hatch and pupation, as a precise count at those stages of development was impractical; handling newly emerged 1st instars during preliminary experiments led to high mortality rates, and disturbing larvae that are ready to pupate may delay pupation (Anderson 2000). The figure of 10% was considered reached for 2nd and 3rd instar stages when 1 individual in a representative

sample of 10 had reached that stage. At each examination time, if any adults were present, they were removed and their number was recorded. Once there was no more adult emergence from a jar, the time of 10% adult emergence was retroactively determined.

Once it was determined that 10% of the population in a particular jar had reached the next stage of development, the previous time when the jar had been checked was used as the minimum time to reach that stage. This was done in order to accurately record all development times as minimum, for the sake of being conservative. After Experiment 1 was fully completed, the mean temperature from oviposition to the emergence of 10% of adults was determined as 19.7 °C +/- SE of 0.1 °C.

***Experiment 2***

The goal of Experiment 2 was to determine the same minimum development times for *P. terraenovae*, but at a constant temperature that was comparable to the overall mean temperature established in Experiment 1. All experimental materials and methods were kept exactly the same as in Experiment 1, except Experiment 2 was conducted under laboratory conditions.

In Experiment 2, all four jars were placed inside an incubator (Conviron® model E7/2) at Simon Fraser University, in Burnaby, British Columbia. The incubator was set to maintain a constant temperature of 20 °C +/- 0.1°C. Actual temperature was recorded every 3 minutes, and never varied more than +/- 0.1°C from the setting. The lights in the incubator were set to be on from 0600 hrs until 1900 hrs, in order to mimic the natural light experienced in Experiment 1. The relative humidity in the incubator was set to 75 % +/- 5 %.

Once Experiment 2 was fully completed, the minimum times to reach each of the five stages of development were compared to those from Experiment 1. In order to make a valid comparison, accumulated degree hours (ADH) were first calculated for each stage of development for both experiments (Anderson 2000; Greenberg 1991). For both Experiment 1 and 2, the number of ADH obtained in the three experimental jars were compared with the control jar using a 1-Sample t-Test (Minitab®). Then, ADH calculated for each of the five development stages were compared between experiments, using a 2-Sample t-Test (Microsoft Excel®).

## RESULTS

For both experiments, there was no difference between the ADH calculated for reaching 1$^{st}$ instar, pupae, and adult stages in the three experimental jars and the control jar ($P < 0.05$). Therefore, ADH from the experimental jars and the control jar were pooled for those three stages of development.

For Experiment 1, mean temperature during the different development stages ranged from 17.5 ° C to 21.2 °C, with an overall mean temperature of 19.7 °C +/- SE of 0.1 °C (Table 1, Figure 1). Temperature fluctuations recorded during Experiment 1 are presented in Figure 1. For both experiments (for all five stages of development), the age and ADH of each jar were calculated (Table 1-2). The mean of the ADH for the three jars (or four jars where the control was included), was also determined (Table 3).

The mean number of ADH recorded at each stage of development were compared between Experiments 1 and 2 (Table 4). *P. terraenovae* raised under a constant temperature regime reached 1st instar, 2nd instar, and 3rd instar stages of development faster than those raised under fluctuating temperatures ($P = 0.016$, $t = 3.99$, $df = 4$; $P = 0.007$, $t = 6.79$, $df = 3$; $P = 0.043$, $t = 2.92$, $df = 4$). No significant differences in the number of ADH at pupae and adult stages were found ($P < .05$).

## DISCUSSION

Crime scenes are often located outdoors, where they are exposed to fluctuating temperatures (Anderson 1995). When using blow fly development to establish minimum time since death, as is often done (Grassberger and Reiter 2002; Marchenko 2001; Anderson 2000; Anderson 1995; Davies and Ratcliffe 1994; Greenberg 1991), care must be taken when applying results obtained in the laboratory to conditions outdoors in a natural environment. This study showed that *P. terraenovae* raised under a constant temperature regime develop faster at the early stages than do those raised under naturally fluctuating conditions. In this case, using developmental data obtained under fixed temperature conditions could have led to an underestimate of the post mortem interval.

An earlier study done on *P. terraenovae* by Davies and Ratcliffe (1994) found that fluctuating temperatures actually led to significantly faster rates of development ($P < .01$), opposite to what was found in our study. Although the methods employed in these two studies were different, the conflicting results suggest more research should be done in this area.

Besides making a direct comparison between developmental times under a fixed temperature versus a fluctuating temperature, our study has also generated useful information about *P. terraenovae.* The data generated here on the rate of development under a constant temperature of 20 °C adds to the limited body of knowledge available. Our study found minimum developmental times that were similar to, but somewhat faster than, those recorded by either Grassberger and Reiter (2002) or Marchenko (2001).

## RECOMMENDATIONS

1. A standard method of gathering developmental data would be useful when trying to compare data obtained by different researchers.
2. The method of determining when a particular larval stage is reached should be consistent between researchers. Standards should be developed for some of the current methods of determining development rates, which include the number of posterior spiracular slits, length measurements, and mass.
3. Determination of 'minimum time' to reach a developmental stage should be consistent between studies, and a standardization method should be developed.

## ACKNOWLEDGMENTS

This work was funded by the Canadian Police Research Centre, Ottawa, Ontario. We would particularly like to thank Ms. Julie Graham for her support and encouragement throughout this research. In addition, we would like to thank the Vancouver Foundation and the Animal Welfare Foundation of Canada for supporting this research.

## REFERENCES


Anderson G.S. Minimum and Maximum Development Rates of Some Forensically Important Calliphoridae (Diptera). J. Forensic Sci. 2000; 45: 824-832.

Anderson G.S. The Use of Insects in Death Investigations: An Analysis of Cases in British Columbia Over a Five Year Period. Can. Soc. Forensic Sci. J. 1995; 28: 277-292.

Clarkson C.A., Hobischak N.R. and Anderson G.S. A comparison of the development rate of *Protophormia terraenovae* (Robineau-Devoidy) raised under constant and fluctuating temperature regimes. Can. Soc. Forensic Sci. J. 2004; 37(2):.

Davies L. and Ratcliffe G.G. Development rates of some pre-adult stages in blowflies with reference to low temperatures. Medical and Veterinary Entomol. 1994; 8: 245-254.

Grassberger M. and Reiter C. Effect of temperature on development of the forensically important holarctic blow fly *Protophormia terraenovae* (Robineau-Desvoidy) (Diptera: Calliphoridae). Forensic Science International. 2002; 128: 177-182.

Greenberg B. Flies as Forensic Indicators. J. Medical Entomol. 1991; 28: 565-577.

Greenberg B. and Tantawi T.I. Different Developmental Strategies in Two Boreal Blow Flies (Diptera: Calliphoridae). J. Medical Entomol. 1993; 30: 481-484.

Kamal A.S. Comparative Study of Thirteen Species of Sarcosaprophagous Calliphoridae and Sarcophagidae I. Bionomics. Annals Entomol. Soc. Am. 1958; 51: 261-270.

Marchenko M.I. Medicolegal relevance of cadaver entomofauna for the determination of the time of death. Forensic Sci. International. 2001; 120: 89-109.

Meidinger D. and Pojar J. Ecosystems of British Columbia. British Columbia Ministry of Forests Research Branch.1991.

Ratte H.T. Temperature and Insect Development. In: Hoffman K.H., Editor, Environmental Physiology and Biochemistry of Insects, Berlin Heidelberg: Springer Verlag, 1984; 33-66.

Table 1. *Temperature data recorded during Experiment 1 (fluctuating temperatures). All temperatures are in degrees Celsius. Temperatures given are those recorded up until the development stage is first reached.*

| **Jar** | **Stage** | **Age (D/H/M)** | **Age (h)** | **Mean temp** | **Min temp** | **Max temp** | **ADH** |
|---|---|---|---|---|---|---|---|
| | | | | | | | |
| 1 | 1st instar | 1/17/55 | 41.9 | 17.6 | 15.5 | 26 | 738.2 |
| 1 | 2nd instar | 4/8/55 | 104.9 | 17.7 | 14.5 | 26 | 1860.2 |
| 1 | 3rd instar | 5/20/55 | 140.9 | 18.9 | 14.5 | 29 | 2663.4 |
| 1 | Pupae | 9/12/55 | 228.9 | 21.1 | 14.5 | 33.5 | 4834.8 |
| 1 | Adult | 19/19/55 | 475.9 | 19.7 | 14 | 33.5 | 9356.6 |
| | | | | | | | |
| 2 | 1st instar | 1/10/55 | 34.9 | 17.8 | 16 | 26 | 621.6 |
| 2 | 2nd instar | 4/8/55 | 104.9 | 17.7 | 14.5 | 26 | 1860.2 |
| 2 | 3rd instar | 5/20/55 | 140.9 | 18.9 | 14.5 | 29 | 2663.4 |
| 2 | Pupae | 9/12/55 | 228.9 | 21.1 | 14.5 | 33.5 | 4832.5 |
| 2 | Adult | 19/15/55 | 471.9 | 19.7 | 14 | 33.5 | 9287.4 |
| | | | | | | | |
| 3 | 1st instar | 1/10/55 | 34.9 | 17.8 | 16 | 26 | 621.6 |
| 3 | 2nd instar | 3/23/55 | 95.9 | 17.5 | 14.5 | 26 | 1681.5 |
| 3 | 3rd instar | 5/16/55 | 136.9 | 18.7 | 14.5 | 29 | 2557.3 |
| 3 | Pupae | 8/13/55 | 205.9 | 21.2 | 14.5 | 33.5 | 4365.5 |
| 3 | Adult | 18/19/55 | 451.9 | 19.8 | 14 | 33.5 | 8929.9 |
| | | | | | | | |
| Control | 1st instar | 1/10/55 | 34.9 | 17.8 | 16 | 26 | 621.6 |
| Control | Pupae | 9/4/55 | 220.9 | 21.2 | 14.5 | 33.5 | 4683.5 |
| Control | Adult | 19/19/55 | 475.9 | 19.7 | 14 | 33.5 | 9356.6 |

Table 2. *Temperature data recorded during Experiment 2 (constant temperature of 20 °C).*

| **Jar** | **Stage** | **Age (D/H/M)** | **Age (h)** | **ADH** |
|---|---|---|---|---|
| | | | | |
| 1 | 1st instar | 1/4/15 | 28.25 | 565 |
| 1 | 2nd instar | 2/21/15 | 69.25 | 1385 |
| 1 | 3rd instar | 5/6/15 | 126.25 | 2525 |
| 1 | Pupae | 11/14/15 | 278.25 | 5565 |
| 1 | Adult | 20/2/15 | 482.25 | 9645 |
| | | | | |
| 2 | 1st instar | 1/1/15 | 25.25 | 505 |
| 2 | 2nd instar | 2/17/15 | 65.25 | 1305 |
| 2 | 3rd instar | 5/6/15 | 126.25 | 2525 |
| 2 | Pupae | 9/17/15 | 233.25 | 4665 |
| 2 | Adult | 18/14/15 | 446.25 | 8925 |
| | | | | |
| 3 | 1st instar | 1/1/15 | 25.25 | 505 |
| 3 | 2nd instar | 2/21/15 | 69.25 | 1385 |
| 3 | 3rd instar | 5/2/15 | 122.25 | 2445 |
| 3 | Pupae | 10/17/15 | 257.25 | 5145 |
| 3 | Adult | 19/2/15 | 458.25 | 9165 |
| | | | | |
| Control | 1st instar | 1/1/15 | 25.25 | 505 |
| Control | Pupae | 9/17/15 | 233.25 | 4665 |
| Control | Adult | 19/21/15 | 471.25 | 9425 |

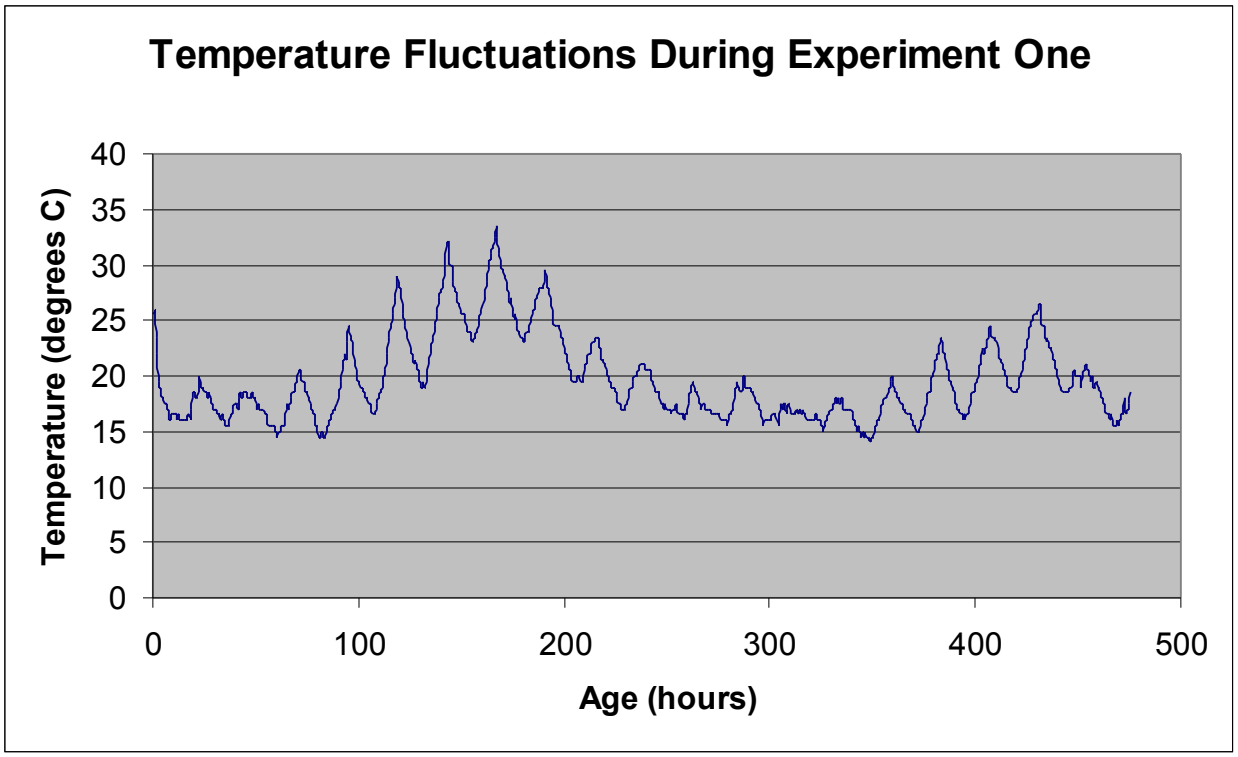
TABLE 3. *Accumulated degree hours (ADH) +/- standard error when each development stage is first reached (Adopted from Clarkson et al. 2004).*

| Experiment 1 (fluctuating temperature) | | Experiment 2 (constant temperature) | |
|---|---|---|---|
| Development Stage Reached | ADH | Development Stage Reached | ADH |
| 1st instar | 650.7 +/- 29.2 | 1st instar | 520.0 +/- 15.0 |
| 2nd instar | 1800.8 +/- 59.4 | 2nd instar | 1358.3 +/- 26.7 |
| 3rd instar | 2627.9 +/- 35.5 | 3rd instar | 2498.3 +/- 26.7 |
| Pupae | 4679.3 +/- 110.4 | Pupae | 5010.0 +/- 216.9 |
| Adult | 9232.6 +/- 102.2 | Adult | 9290.0 +/- 156.3 |

TABLE 4. *Results of a t-Test analysis to determine if there are any differences in development rates for P. terraenovae raised under fluctuating and constant temperatures, at any of the five stages of development.*

| **Stage** | **P** | **t** | **df** |
|---|---|---|---|
| 1st instar | 0.016 | 3.99 | 4 |
| 2nd instar | 0.007 | 6.79 | 3 |
| 3rd instar | 0.043 | 2.92 | 4 |
| Pupae | 0.2546 | -1.36 | 4 |
| Adult | 0.771 | -0.31 | 5 |

Figure 1. *Temperature fluctuations recorded during Experiment One.*


Temperature Fluctuations During Experiment One
Temperature (degrees C)
40
35
30
25
20
15
10
5
0
0
100
200
300
400
500
Age (hours)