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Establishing Lower Developmental Thresholds for a Common BlowFly: For Use in Estimating Elapsed Time since Death Using Entomologyical Methods

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Defence Research and Development Canada
Recherche et développement pour la défense Canada

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Abstract

Forensic entomology is a science used to estimate a post-mortem interval (PMI). Larvae develop at predictable rates and the time interval for this development can be used to estimate the PMI. Environmental temperatures are not constant and fluctuate with the photoperiod. In early and late insect seasons, temperatures can drop to below threshold temperatures where development essentially ceases. Threshold temperatures differ for different species and should be determined by raising insects at the extremely low temperatures. The lower threshold temperature for *Protophormia terraenovae* for egg hatch is 10.3˚C ; for first instar larvae to molt to second instar larvae it is is 10.7˚C ; it falls between 10.7 and 11 ˚C to reach third instar, and is 11 ˚C to reach post feeding third instar larvae. In order to pupate, the temperature threshold falls between 11.5 and 11.7 ˚C and adult emergence is completed only at temperatures above 11.7˚C.

It was shown that if P. *terraenovae* was raised at a higher temperature for a period of time and only exposed to temperatures below thresholds at a later larval stage, development could continue.

Résumé

L'entomologie médico-légale est une discipline scientifique qui permet d'estimer un délai post-mortem. La durée du développement larvaire des insectes est prévisible et peut être utilisée pour estimer le délai post-mortem. En milieu naturel, la température n'est pas constante et fluctue en fonction de la photopériode. Au début et à la fin de la période de développement des insectes, la température peut chuter sous le seuil thermique minimal en deçà duquel le développement est complètement inhibé. La valeur précise de ce seuil diffère d'une espèce d'insecte à une autre et doit être déterminée dans le cadre d'élevages réalisés à très faible température. Chez le *Protophormia terraenovae*, les seuils thermiques minimaux varient selon le stade de développement de l'insecte et s'établissent comme suit : 10,3 °C pour l'éclosion des œufs, 10,7 °C pour la mue du premier au deuxième stade larvaire, entre 10,7 et 11 °C pour la mue du deuxième au troisième stade larvaire, 11 °C pour l'atteinte du stade prépupal, entre 11,5 et 11,7 °C pour la pupaison et 11,7 °pour l'émergence des adultes.

Chez le *P. terraenovae*, il a été démontré que le développement peut se poursuivre normalement si les larves sont maintenues à une température plus élevée avant d'être exposées seulement à la fin de leur développement à une température inférieure au seuil thermique.

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Executive summary

Establishing Lower Developmental Thresholds for a Common BlowFly: For Use in Estimating Elapsed Time since Death Using Entomologyical Methods

Anderson, Gail; Warren,Jodie; DRDC CSS CR 2011-23; Defence R&D Canada – CSS; October 2011.

Introduction or background: Forensic entomology is the study of insects associated with a dead body usually to estimate the elapsed time since death, along with other key factors. The understanding and familiarity of this science has improved dramatically in recent years, with its increased application to death investigations, even though the science has been in use in Europe since the 1850's. Forensic entomology is now an integral part of a death investigation when estimating a time since death beyond 72 hours. Forensic Entomology is considered to be the most accurate method for estimating elapsed time since death, particularly when more than three days have elapsed (Dillon & Anderson, 1995; Kashyap & Pillai, 1989)).

There are two entomological techniques used to estimate elapsed time since death. The first technique is to look at the predictable successional colonization of insects on the body. This method is reserved for a greater elapsed time since death and is usually used when the victim has been dead for four weeks to a year or more. The second technique, however, is applied when death is more recent (hours to weeks). This method involves applying blow fly larval development times to investigations. This area of forensic entomology requires a more thorough examination as most of the research in Canada is devoted to the first technique.

Insect development is predictable but, as insects are cold blooded, ambient temperature has an overwhelming effect on insect development (Andrewartha & Birch, 1954; Chapman, 1982; Wells & Lamotte, 2010). In fact, insect development does not proceed below or above certain "threshold" temperatures (Greenberg & Kunich, 2002; Warren, 2006). In Canada, the lower developmental threshold is most important, as temperatures rarely exceed the upper developmental threshold for very long. As well, although most literature pertains to constant temperatures, in reality of course, temperatures fluctuate. Of particular concern are situations where temperatures fluctuate dramatically such as those seen in spring and fall in Canada, where night time lows can drop to 4-5^{o}C, but day time highs can reach the 20's^{o}C. This came to the fore in a cold case, in which a 16 year old girl was raped and murdered in October 1978 (R. v. Larson), and the case re-examined decades later. Temperature extremes ranged from 2^{o}C to 21^{o}C and made interpretation difficult. In our earlier work (Warren, 2006), we examined development at extreme fluctuations of 4 to 28˚C and 9 to 23˚C; temperatures which may be observed in these early and late insect seasons and compared that development to their mean constant temperature average temperature 16˚C and found surprising development differences.

Sommaire

Établissement d'un seuil thermique minimal pour le développement d'une mouche de la viande commune à des fins d'estimation du délai post-mortem à l'aide de méthodes entomologiques

Gail S. Anderson; Jodie-A. Warren; RDDC CSS CR 2011-23; R & D de la défense Canada – CSS; octobre 2011.

Introduction ou contexte : L'entomologie médico-légale est une discipline scientifique qui se fonde sur l'étude des insectes associés aux cadavres pour établir le délai post-mortem et d'autres facteurs clés. Même si son application en Europe remonte aux années 1850, ce n'est qu'au cours des dernières années qu'elle s'est développée véritablement pour s'imposer comme une discipline scientifique à part entière. Elle est aujourd'hui couramment utilisée pour estimer le délai post-mortem dans le cadre d'enquêtes criminelles portant sur des décès remontant à plus de 72 heures. L'entomologie médico-légale est considérée comme la méthode la plus précise pour estimer le temps écoulé depuis un décès, en particulier si ce dernier date de plus de trois jours (Dillon et Anderson, 1995; Kashyap et Pillai, 1989).

Deux méthodes entomologiques permettent d'estimer le temps écoulé depuis un décès. La première consiste à examiner les étapes successives et prévisibles de la colonisation des cadavres par les insectes. Cette méthode est normalement réservée aux décès qui remontent à plus de quatre semaines à un an. La deuxième méthode est toutefois appliquée aux décès plus récents (quelques heures à quelques semaines). Cette deuxième méthode repose sur l'application des temps de développement larvaire des mouches de la viande aux enquêtes. Une plus grande attention doit être accordée à cette méthode, car la plupart des recherches entreprises au Canada sont consacrées à la première technique.

Le développement des insectes est prévisible, mais comme les insectes sont des organismes à sang froid, sa durée varie en fonction de la température ambiante (Andrewartha et Birch, 1954; Chapman, 1982; Wells et Lamotte, 2010). Le développement des insectes est inhibé en decà ou au-delà de seuils thermiques précis (Greenberg et Kunich, 2002; Warren, 2006). Au Canada, c'est le seuil thermique minimal qui est le plus important, car les températures dépassent rarement le seuil thermique maximal durant une très longue période. Paradoxalement, bien que la température fluctue constamment en milieu naturel, la plupart des études publiées ont été effectuées en présence de températures constantes. Au Canada, l'amplitude des fluctuations journalières de température peut être considérable, en particulier au printemps et en automne, alors que la température peut chuter à 4 ou 5 ^{o}C la nuit pour s'élever à plus de 20 °C le jour. Cette particularité est revenue à l'avant-plan dans une affaire classée de viol et de meurtre (R. *c*. Larson) survenue en octobre 1978. La victime était âgée de 16 ans. L'enquête a été rouverte des décennies plus tard. La forte amplitude des fluctuations de température (de 2 à 21^{o}C) a rendu l'interprétation difficile. Dans le cadre de travaux antérieurs (Warren, 2006), nous avons examiné le développement en présence de fluctuations extrêmes de températures de 4 à 28 ˚C et de 9 à 23 ˚C. De tels écarts de température sont fréquents au début ou à la fin de la période de développement des insectes. Une comparaison des durées de développement observées aux valeurs moyennes enregistrées à une température moyenne de 16˚C a révélé des différentes surprenantes.

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1 Introduction

Forensic entomology is the study of insects associated with a dead body usually to estimate the elapsed time since death, along with other key factors. The understanding and familiarity of this science has improved dramatically in recent years, with its increased application to death investigations, even though the science has been in use in Europe since the 1850's. Forensic entomology is now an integral part of a death investigation when estimating a time since death beyond 72 hours. Forensic Entomology is considered to be the most accurate method for estimating elapsed time since death, particularly when more than three days have elapsed (Dillon & Anderson, 1995; Kashyap & Pillai, 1989)\). [1]

There are two entomological techniques used to estimate elapsed time since death. The first technique is to look at the predictable successional colonization of insects on the body. This method is reserved for a greater elapsed time since death and is usually used when the victim has been dead for four weeks to a year or more. The second technique, however, is applied when death is more recent (hours to weeks). This method involves applying blow fly larval development times to investigations. This area of forensic entomology requires a more thorough examination as most of the research in Canada is devoted to the first technique.

The list of cases in which forensic entomology has helped is innumerable, but at least 200 cases have been analyzed in Canada using data generated out of this class of research. This includes such high profile cases as the Mindy Tran homicide in Kelowna, B.C. which was the instigator for burial research[2] (VanLaerhoven, 1997; VanLaerhoven & Anderson, 1996, 1999), the Louise Ellis homicide and more recently the Robert Pickton homicides. Research regarding submergence times became instrumental to the Green River Task Force who required information on bodies dumped in water[3] (Hobischak, 1997; Hobischak & Anderson, 1999, 2002). CPRC funds have also funded research with VENUS [4](The Victoria Experimental Network Under the Sea) which has benefited cases involving submerged victims. Awareness of the elapsed time since death (also elapsed time since submergence, colonization or freezing etc.) can assist in victim and criminal identification as well as suspect elimination when alibis cannot be ruled out[5] (Catts, 1990; Geberth, 1996; Wells & Lamotte, 2010). It is clear that this research has a major and direct benefit to law enforcement and the police community in general. It is also of scientific merit which is important as this research must be peer reviewed in recognized journals to support it in court.

Insect development is predictable but, as insects are cold blooded, ambient temperature has an overwhelming effect on insect development [6](Andrewartha & Birch, 1954; Chapman, 1982; Wells & Lamotte, 2010). In fact, insect development does not proceed below or above certain "threshold" temperatures[7] (Greenberg & Kunich, 2002; Warren, 2006). In Canada, the lower

[1] (Dillon & Anderson, 1995; Kashyap & Pillai, 1989)
[2] (VanLaerhoven, 1997; VanLaerhoven & Anderson, 1996, 1999)
[3] (Hobischak, 1997; Hobischak & Anderson, 1999, 2002)
[4] (The Victoria Experimental Network Under the Sea)
[5] (Catts, 1990; Geberth, 1996; Wells & Lamotte, 2010)
[6] (Andrewartha & Birch, 1954; Chapman, 1982; Wells & Lamotte, 2010)
[7] (Greenberg & Kunich, 2002; Warren, 2006)

developmental threshold is most important, as temperatures rarely exceed the upper developmental threshold for very long. As well, although most literature pertains to constant temperatures, in reality of course, temperatures fluctuate. Of particular concern are situations where temperatures fluctuate dramatically such as those seen in spring and fall in Canada, where night time lows can drop to 4-5^{o}C, but day time highs can reach the 20's^{o}C. This came to the fore in a cold case, in which a 16 year old girl was raped and murdered in October 1978 (R. v. Larson), and the case re-examined decades later. Temperature extremes ranged from 2^{o}C to 21^{o}C and made interpretation difficult. In our earlier work (Warren, 2006), we examined development at extreme fluctuations of 4 to 28˚C and 9 to 23˚C; temperatures which may be observed in these early and late insect seasons and compared that development to their mean constant temperature average temperature 16˚C and found surprising development differences.

Also in our earlier work[8] (Warren, 2006) it was noted that minimum developmental thresholds vary depending on instar and time within instar. This makes sense as physiological changes occurring during the development of the immature insect change over time, which may make the animal more or less sensitive to temperature. As well, behavioural changes such as larval aggregates can change the effect that temperature may have on the insect and increase the insect development time[9] (Hanski, 1976; Levot, *et al.*, 1979; Wells & Lamotte, 2010) whereas some researchers have reported findings that indicate extremely slowed development at extremely low temperatures, rather than complete cessation [10](Davies & Ratcliffe, 1994).

Some initial work on insect development times related to constant temperatures has been completed, which is the basis for most of the present case analyses[11] (Anderson, 2000; Warren, 2006). Also, some early work on fluctuating temperatures[12] (Clarkson, Hobischak, & Anderson, 2004, 2005) in outdoor settings and in the controlled laboratory[13] (Warren, 2006) have been completed but further research has not been done on lower thresholds and their effect on extreme fluctuations for *Protophormia terraenovae*[14] (Robineau-Desvoidy) of the Lower Mainland of British Columbia. *Protophormia terraenovae* is a common species in Canada and appears in a majority of our death investigations. There is little research on this species and the application of the development rates of this species from other geographic regions should be avoided as the species development can vary regionally[15] (Grassberger & Reiter, 2001; Reiter & Grassberger, 2002). Grassberger and Reiter (2002) in Europe estimate the lower temperature threshold for *P. terraenovae* to be approximately 9ºC whereas Marchenko (2001) in Russia indicates the lower temperature threshold to be only 7.8ºC. These thresholds conflict with Warren (2006) which indicates that egg eclosion could not occur at a temperature as low as 9.8˚C. This may simply be due to regional behavioural differences such as those observed by Wells and LaMotte (2010) for *Chrysomya rufifacies* (Macquart). Depending on its distribution, *C. rufifacies* has been noted to oviposit on fresh and uninfested carcass remains[16] (O'Flynn & Moorehouse, 1979; Shishido & Hardy, 1969; Wells & Lamotte, 2010) but in other locations does not colonize remains until other

[8] (Warren, 2006)
[9] (Hanski, 1976; Levot, *et al.*, 1979; Wells & Lamotte, 2010)
[10] (Davies & Ratcliffe, 1994)
[11] (Anderson, 2000; Warren, 2006)
[12] (Clarkson, Hobischak, & Anderson, 2004, 2005)
[13] (Warren, 2006)
[14] (Robineau-Desvoidy)
[15] (Grassberger & Reiter, 2001; Reiter & Grassberger, 2002)
[16] (O'Flynn & Moorehouse, 1979; Shishido & Hardy, 1969; Wells & Lamotte, 2010)

larvae are first present [17](Bohart & Gressitt, 1951; Wells & Greenberg, 1994; Wells & Lamotte, 2010; Zumpt, 1965).

Besides observing regional differences of species, we have noted that in many of our more northern cases, insects are clearly developing at temperatures which, according to the literature, are well below the lower threshold. This unusual phenomenon has been observed several times in our cases and requires further exploration.

In order to estimate the post-mortem interval, known developmental data must be converted to thermal units. Accumulated degree days/hours are employed and must be established to work backwards from the time of discovery to the minimum time of death. To determine the, ADD/H's, the equation K=y (T-tL) is applied, where y is the development time, T is the temperature and tL is the lower temperature threshold. Because development is believed to come to a standstill at temperatures below the threshold, it is subtracted from the equation. Often an estimated threshold is used which may be extrapolated from a linear regression [18](Ames & Turner, 2003; Grassberger & Reiter, 2002; Liu et al., 1995)), a curvilinear regression or for simplicity assumed to be 0˚C[8] (Warren 2006). By using inaccurate estimations, development may be assumed to still be occurring when it no longer is and vice versa. This can be detrimental to an investigation by providing an extremely poor estimation of the post-mortem interval; therefore, only a well defined threshold should be used. It is vital that we know if a threshold differs from one region to the next or within regions for that matter. For the Lower Mainland of British Columbia, we know that the lower temperature threshold from oviposition to the adult stage of P. terraenovae falls within 11 and 13˚C based on Warren (2006) but it is vital that this number is more specific for our criminal case investigations.

[17] (Bohart & Gressitt, 1951; Wells & Greenberg, 1994; Wells & Lamotte, 2010; Zumpt, 1965)
[18] (Ames & Turner, 2003; Grassberger & Reiter, 2002; Liu et al., 1995))

2 Purpose

The purpose of this research is to explore *P. terraenovae* development at low temperatures in order to establish a more specific lower threshold temperature and to examine development at fluctuating temperatures below development stage thresholds. These will assist in elapsed time since death estimations in criminal investigations by resolving why our criminal case insects show pronounced development in colder temperatures.

3 Materials and Methods

Wild immature *Protophormia terraenovae* were collected from carcass remains and identified as adults once they had eclosed. They were also collected as adults in inverted cone traps. These traps consist of a hollowed metal canister opened at both ends with a metal mesh insert that is open at its apex[19] (Anderson, 2000; Byrd & Butler, 1996, 1997, 1998). A clear plastic bag is fastened to the top of the canister which balances on cork tops above beef liver bait. The adult flies were attracted to the protein bait and because of their flight patterns became trapped in the metal canister. The collected adult and emerged adult *P. terraenovae* were divided and placed into four separate $(50cm)^3$ rearing cages (Figure 1). These insects make up the *P. terraenovae* stock colony insects whose subsequent generations were used in the experiments. The colonies were provided with sugar cubes, milk powder and water *ad libitum* and maintained at room temperature (~20˚C).

[19] (Anderson, 2000; Byrd & Butler, 1996, 1997, 1998)

Figure 1Protophormia terraenovae stock colonies in theCentre of Forennsic Researchof the Centre for Forensic Research

Beef liver was placed into four black 35mm film canisters which were positioned on their sides in each of the four stock colony cages[20] (Byrd and Butler, 1996, 1997, 1998; Warren 2006). The canisters were left in the cages over night and removed first thing in the morning. The meat and eggs from each canister were then placed into their own 4.5 L wide mouthed jar (Figure 2). Each jar held approximately 125mL of dampened pine sawdust flakes and 150g of fresh beef liver. They were separated by a paper towel interface which would soak up any dripping fluids that may have been released from the liver. The extra liver in the jar prevented desiccation of the eggs and offered a larval feeding medium. Liver was added on a regular basis to the jars to further avoid desiccation and to prevent starvation from becoming a factor in development.

[20] (Byrd and Butler, 1996, 1997, 1998; Warren 2006)

Figure 2 4.5 wide mouth experimental jar

Each of the jars was then placed into environment chambers (Figures 3 & 4). To replicate the ambient environment, Conviron® E7/2 plant chambers were used. These chambers maintained constant humidity (75% RH), temperature and photoperiod in each of the chambers. Lighting was consistently left on because we did not want the changes in light to affect the temperature in each of the chambers.

Figure 3 Conviron E7/2 plant environmental chamber

Figure 4 Experimental trial within the chamber with Smartbutton®datalogger recorders

ACR Systems Inc. Smartbutton® dataloggers were placed into each chamber and used to record the temperatures for each trial. Multiple temperatures were examined for each of the stages. The number of posterior spiracular slits alone was used to determine stage (although crop size and behaviour were also taken into consideration at the post feeding stage). Measurements of length was not factored into stage determination as length has been shown to overlap in stages of development[21] (Greenberg, 1991; Greenberg & Tantawi, 1993) and can readily vary if starvation is a factor[22] (Anderson, 2000). Furthermore, we wished to minimize insect exposure to the warm lights of the microscope and to the temperature outside of the environmental chambers while measuring length as this would defeat the purpose of our research. Jars were added to the constant temperature chambers in order to see if development occurred to the following stages. This continued until it could be determined the lowest temperature at which development would still occur. Jars were rotated in the chambers to account for any minor temperature differences within the chambers.

Chambers were checked once daily and if it appeared as though the development had halted, they were then only checked weekly. Experiments were given up to three or four months in a chamber before they were stopped and it was decided that they were not going to develop further at the low temperature.

[21] (Greenberg, 1991; Greenberg & Tantawi, 1993)
[22] (Anderson, 2000)

4 Results

Lower temperature threshold approximations and ranges were identified for each of the stages (Table 1). A confirmed lower temperature threshold was identified to the adult stage. As was found in Warren[8] (2006) the thresholds differ for the different stages and are lower at the earlier stages.

When temperatures were lower than the threshold temperatures and development had ceased for a substantial period of time, jars were moved to a warmer temperature to see if the insects could be revived, but in all cases, death had occurred.

Table 1 Temperature thresholds for P. terraenovae to each of its stages

Stage	Temperature (˚C)
Eggs hatch	~10.3
First instar moult to second instar	~10.7
Second instar to third instar	$10.7<X\leq11$
3rds to post feeding	~11
Pupation	$11.5\leq X\leq11.7$
Adult emergence	11.7

Although specimens could hatch at 10.3^{o}C, they could not develop to the next stage unless the temperature was at least 10.7^{o}C. In fact, complete development to adulthood could not occur below 11.7^{o}C.

As well as the above data, these experiments generated other more interesting data. In most cases, experiments began with eggs being added to each of the chambers and allowed to develop. This took quite some time at such low temperatures and so we decided to try adding later stages to the cold chambers as well.

At low temperatures, specimens placed in the chamber as eggs would not complete the entire lifecycle; however, if specimens were not exposed to low temperatures until a later stage, they were able to continue developing further.

For example, eggs did not hatch at 10^{o}C, but specimens hatched at room temperature and placed in the chamber as first and second instar at 10^{o}C were able to develop to the pupal stage, although they did not emerge as adults. Larvae were also added to a 9˚C chamber as third instar larvae. These larvae pupated at that extremely low temperature, but did not complete development.

5 Discussion

The lower threshold temperature for *Protophormia terraenovae* for the completion of immature development is 10.7˚C but differs for each stage of immature development. The threshold increases for each stage as development advances. This is likely due to the physiological requirements the insect must meet at each stage. It might be expected that later stages are more tolerant of low temperatures, but this does not appear to be the case when development from hatch to adult occurs at a single constant temperature[8] (Warren 2006).

Accumulated degree hours and days may be incorrectly determined for several different reasons. Maggot masses may form and speed up development[23] (Greenberg & Kunich, 2002). A brief submergence may slow the development[23] (Greenberg & Kunich, 2002). Insects containing drugs develop at different rates from their expected development[23] (Greenberg & Kunich, 2002). Finally, threshold temperatures may be incorrectly assumed [23](Greenberg & Kunich, 2002) or be applied from other regions[23] (Grassberger & Reiter, 2002). When this occurs, error is caused in the accumulated degree days which in turn provides an incorrect PMI[23] (Greenberg & Kunich, 2002).

Threshold temperatures are frequently estimated by linear regressions and not determined by experimental methods[24] (Ames & Turner, 2003; Grassberger & Reiter, 2002; Greenberg & Kunich, 2002; Liu *et al*., 1995; Warren, 2006). The linear regression for *P. terraenovae* substantially underestimated the lower temperature threshold to each stage of development, according to Warren[8] (2006). Due to the sigmoid nature of the graph, using linear regression to estimate the lower threshold will underestimate the actual threshold. As well, applying thresholds

[23] (Greenberg & Kunich, 2002)

[24] (Ames & Turner, 2003; Grassberger & Reiter, 2002; Greenberg & Kunich, 2002; Liu *et al*., 1995; Warren, 2006)

from different geographic regions can add further errors. Marchenko[25] (2001) in Russia found the threshold for *P. terraenovae* to be 7.8˚C which differs by 3.9˚C from our determination of 11.7˚C for *P. terraenovae* in the Lower Mainland of British Columbia.

We found thresholds to differ if experiments began at a stage later than the egg stage which is probably fairly typical in a criminal case where insects are developing at habitually changing ambient temperatures in late Spring and early Fall. This would explain why in our criminal cases, insects are being discovered rather far along in their development when temperatures have been so low. It appears that as long as *P. terraenovae* has been exposed to slightly higher temperatures, they are more resistant to lower temperatures. In a case where temperatures were higher for a brief time and development has had a good start and then temperature drops substantially, the insect seems to be able to continue development. We deduce this based on when the decedent was last seen alive and the temperature records for the area.

In conclusion, estimated lower temperature thresholds and thresholds from different geographical regions should be used with caution. Instead experimental data should be used to determine the actual threshold so that error is not compounded in the accumulated degree day/hour estimation and therefore the post-mortem interval estimation. Furthermore, considerations of substantial temperature change from temperatures above the threshold to temperatures below should be included by the forensic entomologist as this may also affect the post-mortem interval as has been observed in our previous criminal cases.

[25] Marchenko, M. I. (2001)

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Forensic entomology is a science used to estimate a post-mortem interval (PMI). Larvae develop at predictable rates and the time interval for this development can be used to estimate the PMI. Environmental temperatures are not constant and fluctuate with the photoperiod. In early and late insect seasons, temperatures can drop to below threshold temperatures where development essentially ceases. Threshold temperatures differ for different species and should be determined by raising insects at the extremely low temperatures. The lower threshold temperature for *Protophormia terraenovae* for egg hatch is 10.3˚C ; for first instar larvae to molt to second instar larvae it is is 10.7˚C ; it falls between 10.7 and 11 ˚C to reach third instar, and is 11 ˚C to reach post feeding third instar larvae. In order to pupate, the temperature threshold falls between 11.5 and 11.7 ˚C and adult emergence is completed only at temperatures above 11.7˚C.

It was shown that if P. *terraenovae* was raised at a higher temperature for a period of time and only exposed to temperatures below thresholds at a later larval stage, development could continue.

L'entomologie médico-légale est une discipline scientifique qui permet d'estimer un délai post-mortem. La durée du développement larvaire des insectes est prévisible et peut être utilisée pour estimer le délai post-mortem. En milieu naturel, la température n'est pas constante et fluctue en fonction de la photopériode. Au début et à la fin de la période de développement des insectes, la température peut chuter sous le seuil thermique minimal en deçà duquel le développement est complètement inhibé. La valeur précise de ce seuil diffère d'une espèce d'insecte à une autre et doit être déterminée dans le cadre d'élevages réalisés à très faible température. Chez le *Protophormia terraenovae*, les seuils thermiques minimaux varient selon le stade de développement de l'insecte et s'établissent comme suit : 10,3 °C pour l'éclosion des œufs, 10,7 °C pour la mue du premier au deuxième stade larvaire, entre 10,7 et 11 °C pour la mue du deuxième au troisième stade larvaire, 11 °C pour l'atteinte du stade prépupal, entre 11,5 et 11,7 °C pour la pupaison et 11,7 °pour l'émergence des adultes.

Chez le *P. terraenovae*, il a été démontré que le développement peut se poursuivre normalement si les larves sont maintenues à une température plus élevée avant d'être exposées seulement à la fin de leur développement à une température inférieure au seuil thermique.

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Forensic Entomology; Post-Mortem Interval; Time of Death; Blow Fly