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DEVELOPMENT, LIFE HISTORY

Laboratory Development and Field Validation of *Phormia regina* (Diptera: Calliphoridae)

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ABSTRACT Immature blow flies (Diptera: Calliphoridae) collected from decomposing human remains are often used to determine the minimum postmortem interval (PMImin). *Phormia regina* (Meigen) is a common blow fly of cosmopolitan distribution that is often associated in such cases. *P. regina* development at two different cyclic temperatures was examined in this study. A field validation study was conducted to determine the accuracy of applying these data to determine the PMImin. Minimal total development time was 32.52 d at cyclic 14.0 ± 2.0°C and 16.60 d at cyclic 20.5 ± 3.1°C. The minimal larval development was significantly different ($P < 0.05$) across temperatures. Larval development needed 15.5 d at 14.0°C and 7.5 d at 20.5°C. For the validation study, instar, mean, and maximum of length and weight data of the larvae collected in the field were analyzed with data generated from the 20.5°C treatment, as it more closely reflected the field conditions experienced. Accuracy in estimating PMImin, was highly variable depending on the unit of measurement used and instar of *P. regina* collected from the field. Using the oldest instar to estimate a PMImin resulted in ranges that always encompassed the true time of colonization. Accuracy in hours when using measurements units as mean length or weight, and maximal length or weight, varied among the larval instars. In the first instar the greatest overestimation was made with maximal weight while the greatest underestimation was made with mean weight. The most accurate estimate produced with first instars was based on maximal length. In the second instar, there was no overestimation and the greatest underestimation was made with mean weight and the most accurate estimate produced was with maximal length. In the third instar, the greatest overestimation was made with maximal length, and the greatest underestimation was made with mean weight. The estimated time of colonization based on maximal weight was most accurate for third instars.

RESUMEN Los estados inmaduros de moscas verdes colectadas de un cuerpo en descomposición son a menudo usados para determinar el mínimo intervalo postmortem (IPMmin). *Phormia regina* (Meigen) es una mosca verde común de distribución cosmopolita que a menudo está asociada a tales casos. El desarrollo de *P. regina* bajo dos diferentes temperaturas cíclicas fue examinado en este estudio. Un estudio de validación fue conducido para determinar la exactitud de aplicar esos datos cuando se determina un IPMmin. El tiempo de desarrollo total mínimo fue de 32.52d a 14°C ± 2.0°C cíclicos y de 16.60d a 20.5°C ± 3.1°C cíclicos. El desarrollo larval mínimo fue significantemente diferente ($P < 0.05$) entre las temperaturas. El desarrollo larval necesitó 15.5d a 14.0°C y 7.5d a 20.5°C. Para el estudio de validación, el instar, la media y el máximo de los datos de longitud y peso de las larvas colectadas en el campo fueron analizadas con los datos generados del tratamiento a 20.5°C, debido a que esta reflejó más cercanamente las condiciones experimentadas en condiciones de campo. La exactitud en la estimación del IPMmin fue altamente variable dependiendo de la unidad de medición usada y del instar de las muestras de *P. regina* que provenían del campo. Usando el instar más avanzado para estimar el IPMmin, resultó en rangos que siempre compaginaron con el verdadero tiempo de colonización. La exactitud en horas, usando unidades de medición comola longitud o peso medio, y la longitud o peso máximo, varió entre los instares larvales. En el primer instar la mayor sobreestimación se obtuvo usando el peso máximo, mientras que la mayor subestimación fue realizada usando el peso medio. La mejor estimación producida con larvas de primer instar se obtuvo usando la longitud máxima. En larvas de segundo instar no hubo sobreestimaciones, la mayor subestimación se realizó usando el peso medio y la mejor estimación usando la longitud máxima. En el tercer instar, la

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mayor sobreestimación se obtuvo con la longitud máxima y la mayor subestimación fue con el peso medio. El tiempo estimado de colonización basado en el peso máximo fue el más exacto para larvas de tercer instar.

KEY WORDS Calliphoridae, forensic entomology, minimal postmortem interval estimate

The black blow fly, *Phormia regina* (Meigen) (Diptera: Calliphoridae) is commonly collected in temperate regions (Scudder and Cannings 2006, Gennard 2007). It is distributed throughout the United States (Cranston 2008) with the exception of southern Florida (Byrd and Castner 2010). *P. regina* is typically considered a cold weather fly and is most abundant in the spring through fall in central regions of North America and is predominantly collected in the southern of United States during warm winter days (James 1947, Kamal 1958, Zumpt 1965, Pratt et al. 1975, Byrd and Castner 2010).

In the northern United States this fly overwinters as larvae, but in the south, as adults (James 1955, Pratt et al. 1975, Scudder and Cannings 2006). It is also a high-altitude fly and is known to cause myiasis (Erzinçlioğlu 1988, Sabrosky et al. 1989, Byrd and Allen 2001, Byrd and Castner 2010) in sheep and cattle in the southwestern United States (James 1955, Pratt et al. 1975, Scudder and Cannings 2006). Larvae also have been used in wound therapy (Robinson 2005).

This species is an important insect in forensic entomology as it is one of the primary species used to indicate a minimum postmortem interval (PMImin) (Byrd and Allen 2001), or the time of colonization of human remains. In British Columbia, an analysis of death investigations involving entomological evidence over a 5 yr period indicated that *P. regina* was commonly collected from victims found inside houses in urban and rural areas (Anderson 1995, Anderson and VanLaerhoven 1996). *P. regina* can often be the first to arrive and colonize a carcass (Joy et al. 2002). Resulting larvae subsequently constitute the primary decomposing force of carrion. Their development is affected and regulated by abiotic factor, such as temperature, light, humidity, and biotic factors such as tissue type (Kaneshrajah and Turner 2004, Clark et al. 2006, Day and Wallman 2006), and conditions of the tissue (i.e., fresh, dry, or congealed) (Aggarwal 2005). These factors lead to variation in development time and thus impact the accuracy of estimating a PMImin with laboratory data. Studies on *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) demonstrate larval development was faster with larvae completing feeding and dispersing from the food source 31 h earlier and being 2 mm longer when reared on lung compared with liver (Clark et al. 2006); for *Calliphora vicina* (Robineau–Desvoidy) larval development was significantly faster by as much as 2 d when reared on lung, kidney, heart, and brain compared when reared on pig liver (Kaneshrajah and Turner 2004); and in the case of the blow flies *Calliphora augur* (F.) and *Lucilia cuprina* (Wiedemann), larvae fed on sheep liver had a slower development than larvae fed on meat and brain (Day and Wallman 2006).

There are some studies on development of *P. regina* (Kamal 1958, Greenberg 1991, Anderson 2000, Byrd and Allen 2001, Nabity et al. 2006); however, results in development time varied across studies. Consequently, it is difficult to determine which data to apply to a case within a given region to provide the greatest level of accuracy when estimating the PMImin. Some researchers have conducted validation studies to determine the percent error, or accuracy, in estimating the PMImin with their data, as those made by Tarone and Foran (2008) who presented a method for modeling the growth of *L. sericata* and predicted the extent of juvenile fly development, using generalized additive models (GAMS); or like the validation study made by VanLaerhoven (2008), in which published developmental data were used to make estimations of sample age from three hypothetical cases, where the estimations and collecting of evidence were made by course participants and the dates of placement of carcasses were kept confidential by the author until after analysis of the insects collected to avoid any bias because of preexisting knowledge. The objectives of the current study were to produce a development data set for *P. regina* in Texas and validate it.

Materials and Methods

Adult Fly Colony. Adults of *P. regina* were collected from pig carcasses placed in a field in College Station, TX, during the spring of 2010. All adults came from the same area and were captured in a period of 2 h in the same day. The flies were placed in 300 cm^3 cages (Bioquip Products, Rancho Dominguez, CA), held under laboratory conditions and provided with water and a 50:50 mixture of sugar and powdered milk ad libitum. Adult flies were provided 50 g bovine liver as an oviposition substrate. Eggs collected less than 1 h after deposition from the F_1 and F_2 generations were used in the laboratory and field studies.

Laboratory Experimental Design. The development study was conducted in two 136LLVL Percival (Percival Scientific, Inc., Perry, IA) growth chambers set at fluctuating temperatures of 14.0 ± 2.0°C and 20.5 ± 3.1°C, respectively, 75% relative humidity (RH) and a photoperiod of 12:12 (L:D) h. Temperatures were alternated in each chamber to avoid chamber effect on larval development. Onset HOBO U12–006 data loggers (Onset Co., Pocasset, MA) with an Onset TMC6-HD air, water, and soil temperature sensor (Onset Co.) were placed in each growth chamber to record temperature hourly throughout the study.

Approximately 250 blow fly eggs were placed on 250 g bovine liver on 200 g vermiculite in each 950 ml glass jar used. In total, 18 jars were setup and used in the study. Nine jars were placed inside each growth chamber of which seven were sampled over time and

two were not in order to determine if handling impacted larval development. However, only time of adult emergence was recorded for the control jars. Jars were covered with a paper towel held in place with a rubber band and placed in their assigned growth chamber. Eggs in each jar were monitored hourly until hatch was observed.

Observations of larval development in a jar were made every 12 h once egg hatch was observed. Four larvae were sampled for each observation. Larvae sampled were parboiled in hot water and dried with a paper towel before taking measurements. Length for each larva was recorded using a stage scale and a Meiji Techno EMZ-8TR microscope (Meiji Techno America, Santa Clara, CA). Weight of each larva was measured using an Adventure-Pro AV64 Ohaus scale (Ohaus, Pine Brook, NJ). Larval instar was also recorded for each specimen. Jars were randomly placed in each growth chamber after each observation. Corresponding observations for adult emergence were made every 12 h as well.

Field Validation Study. *P. regina* eggs were collected from the colony and partitioned into jars using previously described methods. In total, 30 jars containing liver and vermiculite were inoculated with eggs. Jars were placed in a 120 × 80 × 100 cm mesh cage on a 1 m high table that was always in the shade outside the laboratory. An Onset HOBO U12–006 data logger (Onset Co.) with an Onset TMC6-HD air, water, and soil temperature sensor (Onset Co.) was placed inside the cage to record the daily temperatures.

One jar was randomly pulled from the cage every 12 h. Using methods previously described, length, weight, and instar were recorded for all larvae present in each jar sampled. After each jar was selected, the position of all remaining jars in the cage was randomized.

Statistical Analysis. Two two-factor analysis of variance (ANOVA) without replication were made using Microsoft Office Excel version 2007 (Microsoft Corp., Redmond, WA) to determine differences between minimum mean time spent in each developmental stage and between mean percent time spent in each instar and stages among temperatures ($P \leq 0.05$). The confidence intervals (CIs) and plotting of larval length and weight were made using Microsoft Office Excel version 2007 and the modeling of larval length and weight were made using R Program, version 2.7.2 (R Foundation for Statistical Computing 2008). The CIs were made as described by Wells and Lamotte (1995). A regression for the validation study was made using Microsoft Office Excel version 2007.

Results

The minimal accumulated degree hours (ADH) to reach each developmental instar and stage of development at 14.0 and 20.5°C are compared with those from previous studies in Table 1. A base temperature of 0°C was used because of the minimal temperature for activity was different than in temperatures re-

Table 1. A comparison of minimal ADH (based on mean or range temp) to reach a developmental stage with past studies

Stage	Anderson (2000),[a] 16.1°C mean (±SD)	Byrd and Allen (2001),[a] 15°C range ADH (±SD)	Present study 14°C mean (±SD)	Anderson (2000),[a] 23°C mean (±SD)	Byrd and Allen (2001),[a] 20°C range ADH (±SD)	Byrd and Allen (2001),[a] 25 to 15°C range ADH (±SD)	Present study 20.5°C mean (±SD)
First instar	692.3 (±30.6)	480–1,770 (±330)	704 (±104.7)	494.5 (±20.7)	380–800 (±100)	480–920 (±120)	615 (±130.2)
Second instar	2,559.9 (±177.1)	1,200–2,850 (±420)	1,856 (±183.12)	1,812.4 (±140.3)	720–1,480 (±200)	800–1,840 (±260)	1,282.7 (±186.80)
Third instar[b]	4,197.17 (±407.3)	2,460–4,080 (±405)	3,032 (±284.1)	2,914.1 (±223.1)	1,240–2,040 (±200)	1,600–7,680 (±1520)	1,950.5.5 (±318.0)
Pupae	7,096.88 (±334.9)	5,835–7,590 (±525)	5,816 (±316.6)	5,002.5 (±105.8)	3,760–6,000 (±560)	6,720–9,560 (±720)	4,269.9 (±264.4)
Adult emergence	11,532.43 (±264.04)	10,080–12,600 (±765)	10,928 (±562.7)	8,493.9 (±85.1)	7,160–10,520 (±680)	10,640–13,040 (±620)	8,170.7 (±186.8)

[a] Constant temp.
[b] Third instar combines both feeding and nonfeeding stages
Min ADH = Minimal ADH.

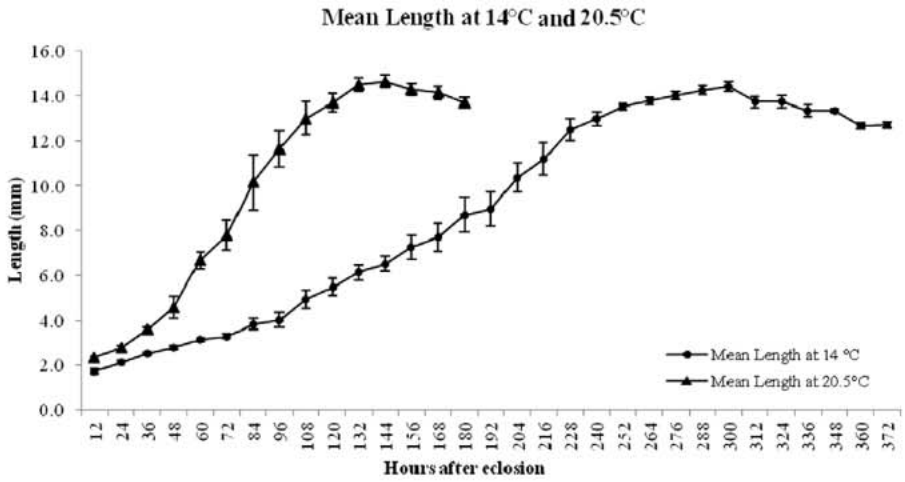

Fig. 1. Mean length development of larval instars of *Phormia regina* reared at cyclic temperatures of 14.0°C (±6°C) and 20.5°C (±6°C). The dark line connects the mean length data.

ported previously by other authors. We found that the time of development differed between temperatures ($F = 8.18$; df = 1; $P < 0.05$). The minimal egg hatching time was 2.0 d at 14.0°C and 1.2 d at 20.5°C. Based on mean of maximal larval length and weight of biggest larvae with 95% CI (Figs. 1 and 2), *P. regina* grew twice as fast at 20.5°C than at 14.0°C. The least amount of time needed to complete larval development after hatching was 7.4 d at 20.5°C and the greatest was 15.2 d at 14.0°C. Minimum time to reach the third instar for larvae reared at 14.0°C was 7 d after hatching. The maximum length at this temperature was 14.6 mm in 12.5 d. Minimum time to reach the third instar for larvae reared at 20.5°C was 3 d after hatching. The maximum length at this temperature was 14.4 mm in 6 d.

Length and weight growth curves were sigmoid shaped indicating that postfeeding larvae were smaller than feeding third instar. Best fit models for length and weight data were fitted with a polynomial and logarithm model, respectively (Fig. 3), as they explain more clearly the changes in length and weight across the development and thus resulted in a more accurate prediction. Models for predicting length and weight individually at each temperature were highly significant, with all cases having an adjusted R^2 of 0.99. In the temperatures of 14.0°C and 20.5°C, the greatest change in maggot length and weight was from 168 to 180 h and from 72 to 84 h, respectively, both ranges were in the ranges of transition from second to third instar. Pupae needed 15.2 and 7.9 d at 14.0 and 20.5°C, respectively. The minimal times from egg to adult emergence at 14.0 and 20.5°C were 32.52 and 16.60 d, respectively.

Because of the field conditions experienced (Fig. 4) during the validation study, the 20.5°C laboratory data set was used to estimate the time of colonization of

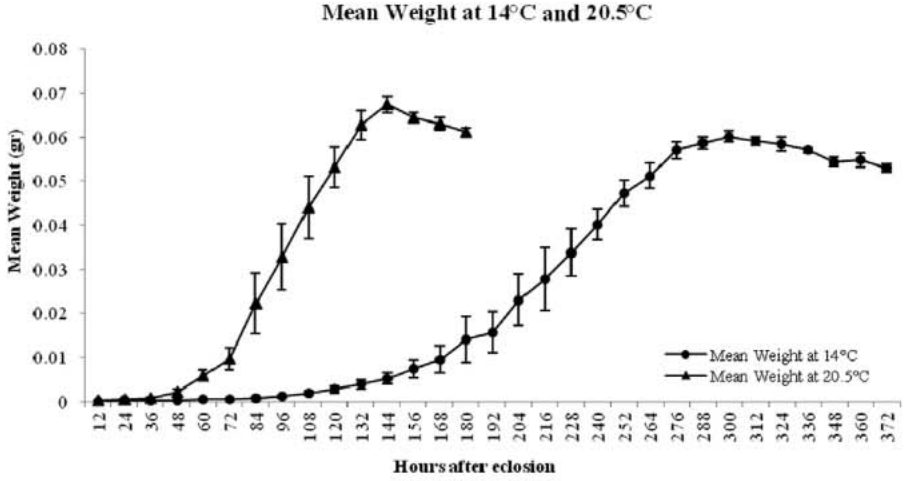

Fig. 2. Mean weight development of larval instars of *Phormia regina* reared at cyclic temperatures of 14.0°C (±6°C) and 20.5°C (±6°C). The dark line connects the mean length data.

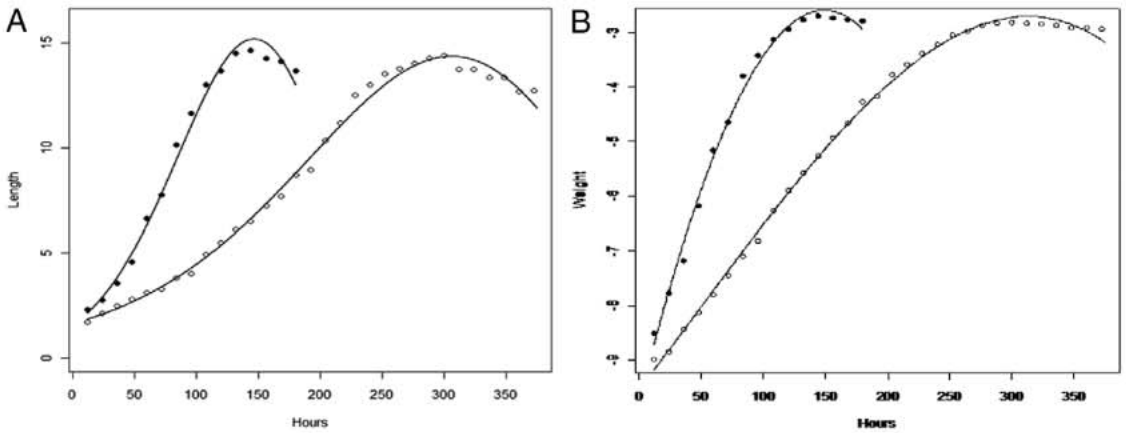

Fig. 3. (A) Length data were fitted with a polynomial model with an adjusted R^2 of 0.9966 at 14°C and 0.992 at 20°C, $\alpha = 0.05$. (B) Weight data fitted with a logarithm model with an adjusted R^2 of 0.998 at 14°C and 0.992 at 20°C, $\alpha = 0.05$. Black points are the data of 20.5°C and white points are the data of 14°C.

each jar sampled from the field. In total, 587 larvae of different ages reared under field conditions were measured. Because the age of the larvae sampled and temperature experienced were known, an inverse prediction was carried out using their mean length and weight, and maximal length and weight.

Other validations were made based on oldest instar present in the sample. In general, all the actual days were inside of the estimated ranges made based on the oldest instar (Table 2). The greatest difference in the PMImin estimated based on larval instar and the actual date was 4 d with larvae that were at the end of third instar and dispersal phase, where the reductions on length and weight occur; and the greatest overestimate from actual date was 2.5 d with larvae that were in the beginning of third instar.

Among general measurements, 71.5% of age estimations were better predicted using the length instead of the weight, and just in 7% of the cases were equal (Fig. 5). When maximal and mean measurements were compared, 64.0% of age estimations were more accurate using maximal measurements and just in 7.0% of estimations were equal. However, a greater variation and likelihood of overestimate of the time of colonization was observed when using maximal measurements in a third instar. When mean length and weight were compared, 71.0% of age estimations were more accurate when the mean length was used and just 14.0% of estimations were equal among mean length and weight. When maximal length and weight were compared, the percentages of accuracy were more equilibrated at 36.0 and 43.0%, respectively. When maximal and mean lengths were compared, 64% of age estimations were more accurate using maximal length instead of mean length with 7.0% of estimations were truly accurate. When maximal and mean weights were compared, 64% of estimations were more accurate when maximal weight was used and 14% of estimations were equal.

Accuracy of estimations made with measurements of each larval instar varied. In first instar, the biggest overestimation was made when using maximal weight

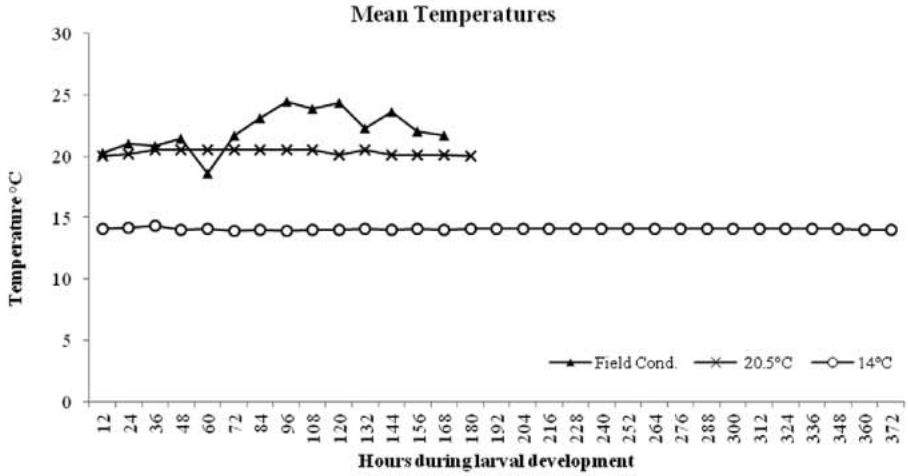

Fig. 4. Mean temperature data for 14.0, 21.5°C, and field conditions through experiments.

Table 2. Comparison of actual PMI (d) with estimated PMImin (d) based on the most advanced instar of development of *P. regina* present in the sample

Jar ([a])	Instar	Actual PMI (d)	Range of estimated PMImin (d)
1 (53)	First	1.5	1–2
2 (9)	First	2	1–2
3 (5)	First	2.5	2.5
4 (4)	Second	3	2–3
5 (28)	Second	3.5	3–4
6 (23)	Second	4	3–4
7 (11)	Third	4.5	4.5
8 (60)	Third	5	3–7
9 (64)	Third	5.5	4–8
10 (39)	Third	6	4–8
11 (55)	Third	6.5	4.5–7.5
12 (47)	Third	7	4–8
13 (53)	Third	7.5	3.5–8.5
14 (22)	Third	8	8

[a] Number of specimens in jar.

and the biggest underestimation was made when using mean weight and the best accuracy was made with maximal length. In second instar, there were no overestimation and the biggest underestimation was made when using mean weight and the best accuracy was with maximal length. In the third instar, the biggest overestimation was made with maximal length, the biggest underestimation was made when using mean weight and the best fit was made with maximal weight. The greatest underestimation from all actual date was 1.3 d using mean weight in third instar and the greatest overestimation was 1.6 d using maximal length in third instar.

Comparing the two laboratory temperatures and field conditions, the percent time spent in each instar and other developmental stages did not differ across temperatures ($F = 2.12$; df = 2; $P = 0.999$; Fig. 6). The validation was made by two ways; first one was made using different variables as: mean length or weight and with maximal length or weight from all larvae per jar and the second one was made on the oldest larval instar present in each jar. The validation was made for the regime of 20.5°C because it more closely reflected the field conditions experienced.

Discussion

As mentioned in other studies (Kamal 1958, Greenberg 1991, Anderson 2000, Byrd and Allen 2001) there was a significant difference in *P. regina* development between temperatures. At a constant temperature of 15°C, Byrd and Allen (2001) reported the egg hatching near to 3.0 d, which is near to our 2.0 d reported in this study at 14°C. Byrd and Allen (2001) also used cyclic and constant temperature of 20°C and Robinson (2005) a temperature of 22°C, both reported hatch time at 1.0 d. Greenberg (1991) reported egg hatch of 20 and 18 h in a constant and cyclic temperature of 22°C, respectively. This is in agreement with our results of hatching in 1.2 d at 20.5°C. Kamal (1958) used a warmer temperature of 26.0°C and despite this; the reported hatching time is shorter than ours at 20.5°C by 5 h. In case of larval development, in the first and second instars, Greenberg (1991) reported that at a constant temperature of 22.0°C the duration was 25 h for each instar, while the duration for the third instar was 113 h, which is very similar to that reported in our study for this instar at 20.5°C. The minimal times to reach the first instar (Table 1) at cyclic temperature of 14.0 and 20.5°C are in agreement with Anderson (2000) who reported that the first instar is reached at 2.0 d at constant temperature of 16.1 and at 23.0°C is reached in 1 d. The minimal times from second instar to pupa reported by Anderson (2000) at 16.1 and 23.0°C were longer than those recorded in this study, but at the end, the adult emergence was in the same time range despite differences in developmental time of previous stages. The onset of larval instars reported

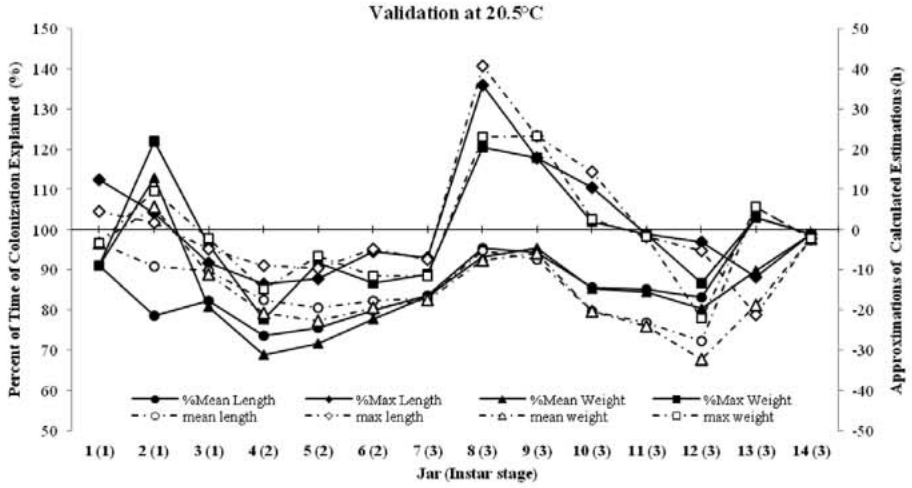

Fig. 5. Percent of time of colonization explained and approximations of calculated estimations to the actual date, based on mean and maximal length and weight of field development. Jar samples (oldest instar present) are listed on the horizontal axis while percent time of colonization explained is on the vertical axis.

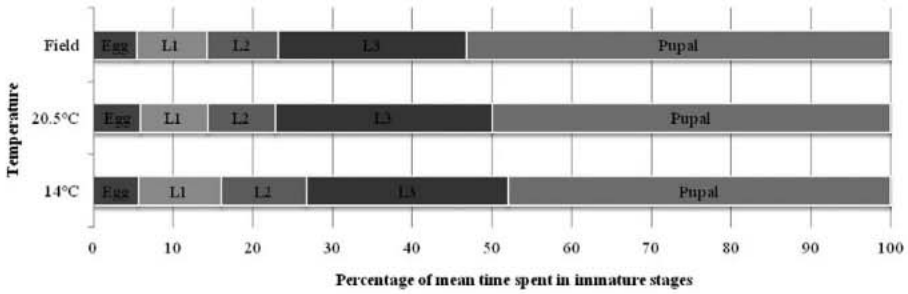

Fig. 6. Percentage of mean time spent in each instar and stages of development of the cycle.

by Byrd and Allen (2001) at 15.0 and 20.0°C occurred faster than both temperatures used in this study, although ours results falls within most of the range established by this author. Robinson (2005) reported that the third instar was completed in ≈3.1 d at 22°C, at our 20.5°C; the larvae reach the third instar at 3.7 d after hatching with a peak development ≈6 d after hatching and a maximum length of 14.4 mm. This maximal size is in agreement with the reported by Robinson (2005), who mentioned that the full grown larva is in the range of 12–17 mm. Most of duration ranges reported in this study are in agree with the ranges reported by Kamal (1958) at 26.0°C, except that our second instar needed more time. Our results at 20.5°C regarding pupal stage and adult emergence are inconsistent with those reported by Byrd and Allen (2001) at a cyclic temperature of 20.0°C, where the onset of pupal stage and adult emergence were delayed 8 and 7 d, respectively, compared with our data. This difference can be because of that Byrd and Allen (2001) have changed the photoperiod from 12:12 (L:D) h during larval development to 24:0 (L:D) h in pupal stage.

Pupal and adult emergence times varied across studies. Greenberg (1991) and Robinson (2005) reported pupal duration when reared at constant 22.0°C was 4.8 d that differed from our results where we determined the pupal stage lasted 8.4 d at 20.5°C and 15.71 d at 14°C. The onset of adult emergence at 14.0°C was 32.52 d since eggs were laid, which is related with reported by Anderson (2000) who mentioned that the minimal adult emergence was at 29.84 d at constant temperature of 16.1°C, while Byrd and Allen (2001) reported the emergence at 28.0 d at constant temperature of 15.0°C. Our adult emergence is in disagrees with Nabity et al. (2006) who reported the emergence in 47.0 d at 14.0°C and 39.0 d at 15.0°C. At 20.5°C our adult emergence time was in 16.6 d, which is in agreement with Anderson (2000) who reported a minimal adult emergence in 15.38 d at 23.0°C, Byrd and Allen (2001) in 14.9 d at constant temperature of 20.0°C, Greenberg (1991) in 15.3 and 15.8 d with constant and cyclic temperature at 22.5°C, respectively; Kamal (1958) in 16.0 d at 26.0°C, but is in disagreement with the reported by Byrd and Allen (2001) at cyclic temperature of 20.0°C and Nabity et al. (2006) at 20.0°C where the emergences were at 21.7 and 19.9 d, respectively. Although, Kamal (1958), used a warmer temperature than used here, our results are very similar, this can be because of the differences in the methodology used, Kamal (1958) used an air conditioner in a insectary room instead of growth chambers to regulate the temperature, which had little control of temperature.

Byrd and Allen (2001) reported that in cyclic temperature at 10.0 to 15.0°C there was no adult emergence; however, in our regime of cyclic temperature at 14.0°C (9.0 to 19.0°C) the development was completed. The differences among studies can be due differences at population level of the same species among different geographical regions. (Tarone and Foran 2006, VanLaerhoven 2008). Other differences can be the handling, amplitude of temperatures used and the time of exposure to low temperatures in the cyclic regimens, photoperiod interval, sampling frequency, among others. Caution should be applied when applying data generated in one geographic region to determinate the PMImin for human remains in a different region.

Few validation studies have been conducted (Tarone and Foran 2008, VanLaerhoven 2008). In our study, there was an increment in imprecision of age estimation as larval development continues and this has been referred in literature (Wells and Lamotte 1995, Tarone and Foran 2008).

VanLaerhoven (2008) made estimations based on larval instars using data sets from different authors and mentioned that estimations made with a threshold of 0°C were the most accurately. The accuracy of our estimations based on oldest instar are in the ranges generated with threshold 0°C, but the accuracy is improved when can be recognized by morphological data, in which part of the stage is the sample (as the molt stage or when using body sizes), if is not known it can wide the range estimations, especially in third instar.

As mentioned by Tarone and Foran (2008), the estimations accuracy when using length or weight in first and second instars is much better than in the third instar, because of the first and second had a shortest developmental period and for this reason those instars had more accuracy in age estimations. Tarone and Foran (2008) reported that the postfeeding third instar was the most inaccurate estimating the age, in our study the most inaccurate was the beginning of third

instar that is when the greatest change in size during larval development occurred.

Tarone and Foran (2008) mention that the length and weight had an accuracy of 63.3 and 65.7%, respectively; however, our estimations with body sizes that did not overestimated the age had a percent of mean accuracy of 85, 92, 84, and 90% using mean length, maximal length, mean weight, and maximal weight, respectively, and the percent of overestimations was 5, 16, 13, and 13% for mean length, maximal length, mean weight, and maximal weight, respectively, been most of the overestimations (69%) in the third instar.

In the third instar the variation on body sizes increased and the accuracy in determining actual time of colonization based on time (hours) decreased. However, the percent of accuracy that was better explained correspond to the first and third instar. In this case the measurements that had a better accuracy in hours and explains better the data were the maximal length followed by maximal weight; however, they can overestimate the age especially in the third instar and the weight can be difficult to estimate in young first instar. However, estimations made with measurements of body had a lower overestimation compared with those estimations made with larval instars. In our case, the overestimations can be because the field temperature during those observations was higher than the total mean field temperature. The CIs were made with a 95% confidence level, so the probability of an error when making an estimate is quite low.

Most studies about larval development just take into account either one unit of measurement or larval instar. Length and weight were better predictors than larval instar; however, we suggest to take into account both measurements and larval instar to increase precision in the estimation of the larval age. It is important to do fixation and measurements (mean or maximal) of the larvae in the same way as was done in this study and the studies we referenced.

In this study the percentage of time spent in each instar and stage of development was very similar between the different temperatures used, which was in agreement with most of the data reported by Anderson (2000). Similarly, the maximum size reached in each instar was similar between the temperatures used in this study.

We believe that the data generated based on developmental time, length, and weight realized in this study can be applied in forensic cases in Texas where *P. regina* exists and the mean temperature can be near to those used in this study. However, because of differences between studies, a standardized protocol should be developed and more studies are needed throughout the seasons, in different regions of Texas and across the United States, to have a better understanding of development and variations of *P. regina*.

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