Rearing container size impacts immature development time of *Phormia regina* (Meigen) (Diptera: Calliphoridae) and time of colonization estimations

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Abstract: Development rate is the primary biological parameter used by forensic entomologists to estimate the time of colonization (TOC). As such, the importance of quantifying the effects of intrinsic and extrinsic factors on development rate, as well as their impact on TOC estimations, cannot be understated. Here, we examined the impact of a potentially important, yet overlooked, component of development study design (rearing container size) on immature development time of the black blow fly, *Phormia regina* (Meigen). To test this, first instar *P. regina* larvae were arbitrarily assigned to one of three container sizes (small, medium, and large) and were observed every 24 h for the post-feeding stage, pupation, and adult eclosion. We observed significantly shorter larval development times ($P < 0.003$) and significantly longer pupal development times ($P < 0.001$) in large containers compared to medium and small containers. Furthermore, we used the data generated from our study along with four additional published developmental studies for *P. regina* to estimate the TOC of four sets of human remains at the Anthropology Research Facility at the University of Tennessee. Our data from all container sizes produced accurate estimations for Donors 1, 3, and 4, while we obtained accurate estimations only from the small treatment for Donor 2. The published datasets for *P. regina* examined here each produced accurate estimation ranges for at least 3 of 4 human donors. Overall, we showed that rearing container size significantly impacts attributes of blow fly development, and that this has the potential to impact the accuracy of TOC estimations with human remains.

Keywords: Rearing container size, development, time of colonization, *Phormia regina*

Introduction

Blow flies (Diptera: Calliphoridae) play a vital role in forensic death investigations as their biological development is reliant on vertebrate decomposition (1). As such, the age of blow fly larvae collected from a death scene can provide a timeline of insect colonization events after death. Using known developmental information for a given species, forensic entomologists can estimate a time of colonization (TOC) and thus infer a minimum postmortem interval (PMI_{MIN}) for remains in a legal investigation (2, 3). However, the accuracy of the TOC estimation is affected by the quality of reference data used to make such an estimation. To achieve the most accurate estimation, it is important to understand the potential sources of error from a given dataset. It is well established that blow fly development rate can vary by species, however numerous abiotic and biotic variables, such as temperature (4-6), photoperiod (7-9), tissue type (10-12), larval density (13, 14),

and competition (15, 16), may also have significant effects. Additionally, forensically important developmental phenotypes have been shown to be intrinsically different between conspecific populations (17-19).

Few studies have explicitly examined the introduction of error due to the type of rearing containers chosen by the researcher (7). As there are currently no standardized methods for conducting development studies, and design is often left to the personal preference of the investigator, it is difficult to determine whether the chosen rearing container design contributes to developmental discrepancies between studies. Thus, specific information regarding the rearing container (e.g., size and volume of the feeding container, size and volume of pupation container, type of pupation substrate used) is highly variable between studies. For example, Anderson (2000) provides useful information regarding the 4.5 L glass jar with ~5 cm dampened sawdust as a pupation substrate in her study (4). Similarly, Nunez-Vasquez et al. (2013) indicated that she used a 950 mL glass jar with 200 g vermiculite as a pupation substrate (20). Conversely, other well-known datasets provide little to no information about the rearing containers used to generate development data. Byrd and Allen (2001) give the diameter and height of the feeding cup used (8.5 cm x 11 cm), but not the container holding the pupation substrate (it is unclear if a separate container housing the pupation substrate was used) (21). Greenberg (1991) gives negligible information about any of the methods used to generate the blow fly development data in his study (1), and though Kamal (1958) provides information regarding rearing substrate, there is no mention of the rearing containers or pupation substrates used in this study (22). To date, comparisons of rearing container size have only been touched on lightly in the published literature. For example, Nabity et al. (2007) compared pupation container size to those used in Nabity et al. (2006) (2.0 L vs. 3.6 L), though no significant differences were observed in development times between studies (7, 23). However, Weidner et al (2014) demonstrated that large rearing containers (56.8 L) encourage pupation in the difficult-to-maintain *Lucilia coeruleiviridis* (Macquart), lending support to the theory that increasing area for dispersal may affect laboratory-generated development data (24). As the rearing container size and volume are crucial aspects of experimental design and can ultimately be decided by personal preference of the researcher or availability of supplies, we argue that differences in the sizes of these containers should be examined as they may contribute to discrepancies between studies.

The purpose of this study is to determine if rearing container size significantly impacts forensically relevant developmental parameters of the black blow fly, *Phormia regina* (Meigen). Though there are standard practices outlined for most aspects of applied forensic entomology (3), there are currently no standardized methods in place for generating development data of forensically important insects. As such, it is crucial to understand all components implemented by the researcher that may inadvertently impact development rate. Furthermore, there is a great need in forensic entomology to validate development data with vertebrate remains (ideally human remains) under field conditions to determine if the dataset actually "works". Validation of developmental datasets is rare (20, 25-29) but it is needed to give credibility to the field of forensic entomology. The first objective of this project was to determine if the size of the rearing container impacts survival, development rate, and size of *P. regina* under controlled laboratory conditions. This is beneficial for general colony maintenance in a laboratory when increased survival, shorter development times, and larger sizes (which infer higher fitness) are desirable. We hypothesized that larger rearing containers would lead to shorter development times, higher survival rate, and larger individuals. The second objective of this project was to test the utility of our larval dataset in estimating the TOC with human remains in the field. This objective included a comparison of TOC estimations using four published developmental datasets generated for *P. regina*. Specifically, we hypothesized that data generated from different size containers would lead to differences in TOC accuracy.

Methods

A colony was established from wild-caught adult and larval *P. regina* from the Anthropology Research Facility (ARF) at the University of Tennessee (UTK) in Knoxville, TN, USA and maintained at the Johnson Animal Research and Teaching Unit (JARTU) on the UTK campus. Temperature and humidity were monitored throughout the experiment with a TinyTag Plus 2 data logger (Gemini Data Loggers, Chichester, West Sussex, UK). The average temperature and relative humidity during the experiment was 30.5 ± 0.5 °C and 22.7 ± 8.8% RH. Colonies were maintained at ambient conditions and given water and table sugar *ad libitum*. To induce oviposition for the current experiment, colonies of G_6 flies were exposed to a Kimwipe™ (Kimberly-Clark Global Sales, Inc., Roswell, GA) soaked in chicken blood and approximately 5 g chicken liver held within an 88.7 mL plastic bath cup (Great Value™ brand, Wal-Mart Stores, Inc., Bentonville, AR). Cups were checked every 3 h for eggs. Once approximately 1000 eggs were observed, cups were removed from the cages and all eggs were consolidated onto 5 g lean pork muscle contained within an 88.7 mL bath cup and covered with a Kimwipe™. Eggs were then left undisturbed for 15 h to hatch.

After this period, N = 9 feeding cups containing 50 g lean pork muscle were inoculated with N = 100 1st instar larvae. Feeding cups were then arbitrarily assigned to one of three experimental container sizes: small, medium, or large (Figure 1). The small treatment consisted of three 0.95 L, 14.0 cm x 8.9 cm x 11.4 cm plastic deli containers (Freshware® brand, Amazon, Seattle, WA) covered with a 15.9 cm x 16.1 cm paper napkin (Vanity Fair® brand, Wal-Mart Stores, Inc., Bentonville, AR) and secured with two rubber bands. The medium treatment consisted of three 5.7 L, 34.6 cm x 21.0 cm x 12.4 cm plastic containers with a plastic lid (Sterilite® brand, Lake Havasu City, AZ). The large treatment consisted of three 39.0 L, 89.9 cm x 42.5 cm x 14.9 cm plastic containers with a plastic lid (Sterilite® brand, Lake Havasu City, AZ). All containers were coated with one layer of Fluon® (Insects-a-Slip; Bioquip, Rancho Domingo, CA) to prevent escape of wandering third instar larvae and then filled with play sand (Quikrete®, Atlanta, GA). As the total amount of sand could not be held constant between treatments due to size differences of the containers, the depth of the sand was instead held constant at 2.54 cm. After inoculation, containers were observed every 24 h for post-feeding third instar larvae. Once post-feeding larvae were observed, sand was sifted with a 20.3 cm diameter stainless steel USA Standard Test Sieve with 1.18 mm mesh (Hogentogler & Co., Inc., Columbia, MD) to check for pupation. Though it is possible that disturbing larvae in the sand may potentially impact development rate, all replicates in this experiment were handled as minimally as possible and in the exact same manner. Once pupation was observed, pupae were collected from each container, weighed using a Scout™ SPX Portable Balance (Ohaus Corporation, Parsippany, NJ), and placed in an individualized 29.6 mL plastic portion cup with a lid (PlastX™ brand, Amazon, Seattle, WA). Pupae were checked daily for adult eclosion, at which point adults were killed via freezing at -20.0°C. Dead adults were thawed, sexed, and weighed to the nearest gram.

Data collected in this experiment included: appearance of post-feeding third instar larvae (h), larval development time (h), pupation time (h), total development time (h), pupal mass (g), adult mass (g), sex, larval survival, and pupal survival. A Kruskal-Wallis test with post-hoc Dunn's test were used to determine any significant differences between treatments as data were not normally distributed (30, 31). Sex was used as a covariate to determine any sex-specific interactions with the treatments. All statistical analyses were performed in RStudio (Version 1.2.1335) using native packages and the package *dunn.test* (32, 33).

To determine the impact that rearing container size may have on applied forensic entomology methods, data from this experiment were used to generate TOC estimations of human remains. Additionally, TOC estimations were generated from data of four published development studies of *P. regina*: Byrd and Allen (2001), Kamal (1958), Greenberg (1991), and Nunez-Vasquez (2013) for comparison to the current study. Estimation ranges from each dataset (consisting of the minimum time to complete the third instar stage and the maximum time to complete the post-feeding third instar (=pre-pupal) stage) were used to estimate the

age of wild post-feeding *P. regina* larvae collected from human remains at the ARF (34). Criteria for specimens collected from human donors included only using post-feeding *P. regina* and only using donors who had experienced a mean temperature above 20.0˚C from the time they were placed to the time when samples were collected, which gave us four total donors. All donors were placed unclothed and supine on the ground surface at the ARF. Donor 1 was placed in April 2019, Donors 2 and 4 were placed in August 2020, and Donor 3 was placed in September 2020. Twice daily observations of Donor 1 allowed for the determination of the TOC within an 8 h period. Continuous surveillance of Donors 2 – 4 following placement allowed for the determination of the exact TOC. Larval samples were collected, killed via submersion in boiling water for 30 s, and preserved in 70% ethanol from each donor throughout decomposition. Larval identifications were made with the aid of a dichotomous key (35). Ambient temperatures recorded from the Tyson-McGhee Airport in Alcoa, TN were obtained from the National Oceanic and Atmospheric Administration (NOAA) for the period spanning from the sampling date to the placement date of each donor (36). Local weather station data was used instead of on-site data to replicate the methods used in an actual forensic case. Temperature data were converted to accumulated degree hours (ADH) using a base temperature of 10.0˚C (37). Published development data given in hours were converted to ADH using a base temperature of 10.0˚C. As the Nunez-Vasquez et al. (2013) study provided ADH using a base temperature of 0.0˚C, these values were converted to hours and then converted back to ADH using a base temperature of 10.0˚C. Accuracy was determined by evaluating TOC estimations against the true TOC of each donor. An estimation was considered accurate when the true TOC ADH was bracketed by the estimated ADH range. An underestimation was determined if the estimated range occurred after the true TOC, whereas an overestimation was determined if the estimated range occurred before the true TOC.

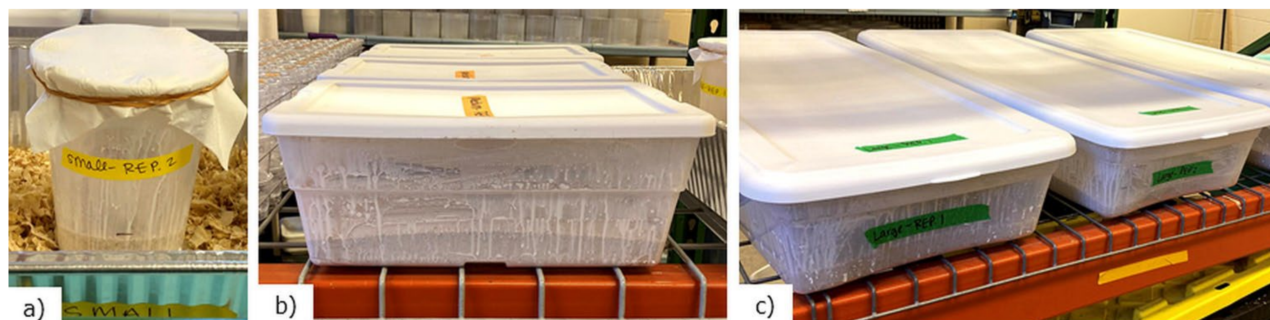

FIGURE 1. Examples of small (a), medium (b), and large (c) rearing containers used in this experiment.

Results

There were no significant impacts of sex on survival, development time, or mass in this experiment ($P > 0.05$). Although not significant, the small treatment exhibited the lowest larval and pupal survival (75% and 46%, respectively), whereas the medium treatment exhibited the highest larval survival (90%) and both medium and large treatments showed similar pupal survival (64%; Figure 2a, Supplemental File 1). Appearance times of post-feeding larvae were not significantly different between treatments ($P > 0.05$; Table 1, Supplemental File 1). Overall, post-feeding larvae were observed between 67.5 h and 216 h after eggs were obtained (Table 1). The Kruskal-Wallis test revealed significant differences in larval development times based on treatment ($\chi^2 = 18.31$, df = 2, $P < 0.001$). The post-hoc paired comparisons revealed significantly shorter larval development times

for the large treatment compared to the small treatment (z = -4.19, $P < 0.001$) as well as to the medium treatment (z = -2.75, P = 0.003; Table 1, Figure 2b, Supplemental File 1). In addition, the pupal development times were significantly different based on treatment (χ^2 = 14.49, df = 2, $P < 0.001$), though the opposite trend occurred. The large treatment exhibited significantly longer pupal development times compared to the small treatment (z = 3.29, $P < 0.001$) and the medium treatment (z = 3.21, $P < 0.001$). However, there was no statistical difference in larval or pupal development times between small and medium treatments ($P > 0.05$). When larval and pupal development times were combined for a total development time, there were no significant differences between the treatments ($P > 0.05$). Pupae and adults from the small treatments were smaller compared to those from the medium and large treatments, though these differences were not statistically significant (Figure 2c, Supplemental File 1).

Data generated from various sized rearing containers led to TOC estimations that were different in terms of their accuracy for Donor 2. In this case, the estimation ranges generated from the medium and large treatments overestimated the true TOC, whereas data from the small treatment encompassed the true TOC (Table 2). For all other donors, the TOC estimations made with data from this study encompassed the initial oviposition time, even though ambient temperatures experienced by the wild-caught larvae were 5 – 10˚C lower than our study temperature (Table 2). Accurate TOC estimations were obtained for all donors when using the Byrd and Allen (2001) and Kamal (1958) datasets. The Nunez-Vasquez et al. (2013) dataset was accurate for all donors except Donor 2, for which the TOC was overestimated. The Greenberg (1991) 22˚C dataset underestimated the TOC for Donor 1, but the 22 and 29˚C datasets from that study accurately reflected the TOC for all other donors.

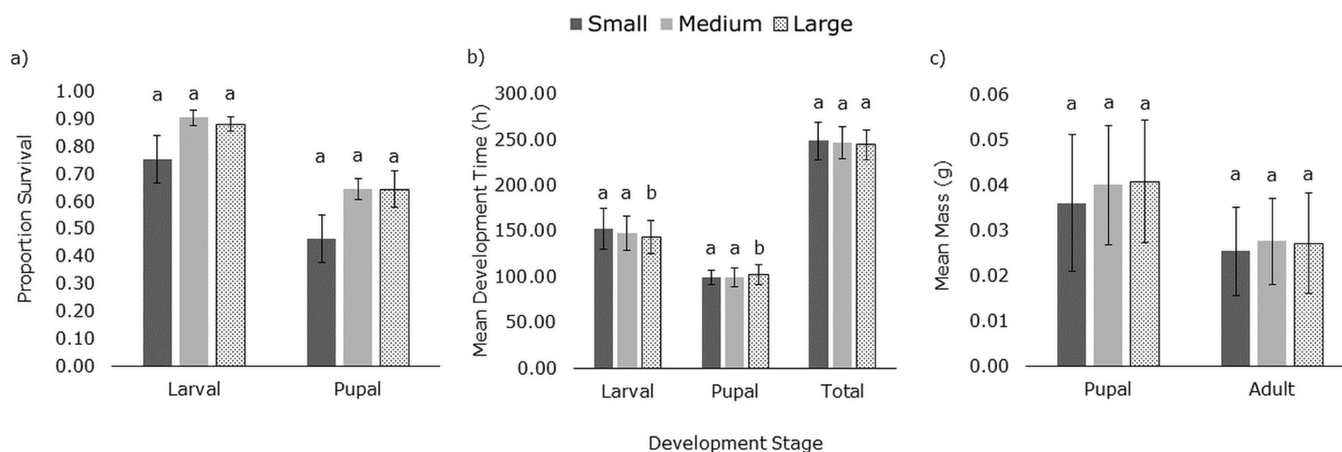

TABLE 1. *Summary of immature development data (mean ± standard deviation, minimum – maximum).*

Treat.	Appearance of PF Larvae		Development Time: Larval		Pupal		Total	
	Hours	ADH	Hours	ADH	Hours	ADH	Hours	ADH
Sm.	*119.6 ± 29.2 (67.5 – 168.0)	2451.6 ± 598.2 (1383.8 – 3444.0)	†152.4 ± 22.8 (120.0 – 216.0)	3123.1 ± 468.2 (2460.0 – 4428.0)	99.1 ± 8.1 (96.0 – 120.0)	2031.7 ± 165.8 (1968.0 – 2460.0)	248.8 ± 20.7 (216.0 – 336.0)	5100.5 ± 423.6 (4428.0 – 6888.0)
Md.	140.3 ± 37.8 (96.0 – 216.0)	2876.3 ± 773.9 (1968.0 – 4428.0)	147.8 ± 18.9 (120.0 – 192.0)	3030.2 ± 388.2 (2460.0 – 3936.0)	99.4 ± 10.0 (72.0 – 120.0)	2036.8 ± 204.7 (1476.0 – 2460.0)	246.1 ± 17.5 (216.0 – 312.0)	5044.9 ± 359.6 (4428.0 – 6396.0)
Lg.	138.5 ± 34.2 (96.0 – 192.0)	2838.5 ± 700.2 (1968.0 – 3936.0)	143.3 ± 17.9 (120.0 – 216.0)	2938.5 ± 367.8 (2460.0 – 4428.0)	102.7 ± 10.8 (96.0 – 120.0)	2105.7 ± 221.4 (1968.0 – 2460.0)	244.4 ± 16.5 (216.0 – 312.0	5009.2 ± 338.1 (4428.0 – 6478.0

*Represents timepoints at which post-feeding third instar larvae were observed for each treatment.
†Represents development times for the larval, pupal, and total immature development stages.

Table 2. *Summary of TOC estimations for four human donors.*

Donor	*Ambient Temp. (˚C)	†TOC (ADH)	Dataset	Temp. (˚C)	Size	Estimation (ADH)	Accuracy
1	20.4	3238.9	Current	30.5	Sm.	1383.8 – 4428.0	Accurate
					Md.	1968.0 – 3936.0	Accurate
					Lg.	1968.0 – 4428.0	Accurate
			Byrd and Allen (2001)	20.0		1360.0 – 4350.0	Accurate
			Kamal (1958)	26.7		1118.9 – 4976.6	Accurate
			Greenberg (1991)	22.0		1140.0 – 2640.0	Underestimation
			Nunez-Vasquez et al. (2013)	20.5		1971.0 – 4158.1	Accurate
2	23.3	1810.3	Current	30.5	Sm.	1383.8 – 4428.0	Accurate
					Md.	1968.0 – 3936.0	Overestimation
					Lg.	1968.0 – 4428.0	Overestimation
			Byrd and Allen (2001)	20.0		1360.0 – 4350.0	Accurate
				25.0		1777.5 – 6510.0	Accurate
			Kamal (1958)	26.7		1118.9 – 4976.6	Accurate
			Greenberg (1991)	22.0		1140.0 – 2640.0	Accurate
			Nunez-Vasquez et al. (2013)	20.5		1971.0 – 4158.1	Overestimation
3	25	2032.5	Current	30.5	Sm.	1383.8 – 4428.0	Accurate
					Md.	1968.0 – 3936.0	Accurate
					Lg.	1968.0 – 4428.0	Accurate
			Byrd and Allen (2001)	25.0		1777.5 – 6510.0	Accurate
			Kamal (1958)	26.7		1118.9 – 4976.6	Accurate
			Greenberg (1991)	29.0		1330.0 – 3420.0	Accurate
			Nunez-Vasquez et al. (2013)	20.5		1971.0 – 4158.1	Accurate
4	25.4	2509.7	Current	30.5	Sm.	1383.8 – 4428.0	Accurate
					Md.	1968.0 – 3936.0	Accurate
					Lg.	1968.0 – 4428.0	Accurate
			Byrd and Allen (2001)	25.0		1777.5 – 6510.0	Accurate
			Kamal (1958)	26.7		1118.9 – 4976.6	Accurate
			Greenberg (1991)	29.0		1330.0 – 3420.0	Accurate

	Nunez-Vasquez et al. (2013)	20.5	1971.0 – 4158.1	Accurate

*Ambient air temperature refers to the mean temperature experienced by each donor from the time of placement to the time of sample collection.

†The TOC for each donor is given as the total ADH acquired from initial oviposition to larval sampling.

Discussion and Conclusion

We have shown that rearing container size has the potential to impact the larval development rate of *P. regina*, and that these differences can impact TOC estimation accuracy. However, the developmental ranges generated in this study overlap with many of the previous datasets generated for this species, and overall, data from several of these studies produced consistently accurate TOC estimations for human remains. Furthermore, we have shown that increasing the rearing container size can lead to higher survival of lab cohorts, as well as slightly larger body sizes, which may correspond to increased fitness of adults. These benefits may be of interest to researchers who maintain laboratory colonies that can be susceptible to unwanted bottlenecks and die-offs.

Though there were many differences in design and implementation of the studies used for TOC comparisons, the importance of validating these datasets in the field cannot be understated. Beyond discrepancies in rearing container size, the type of larval substrate used was different among many of the studies (pork (21, current study), beef liver (20), various substrates (22), and unknown (1)) and could potentially explain differences in development times. Additionally, the minimum temperature thresholds could vary geographically among *P. regina* populations, which may impact the TOC estimation potential of certain datasets. However, we have shown that even given these differences, all datasets were able to accurately estimate the TOC for at least 3 of 4 human donors. Though our human population used for this validation was small (N = 4), the results so far are promising in that it appears that the available datasets for *P. regina* are quite robust.

When comparing the full range of larval development times obtained in the current study to other studies conducted at approximately the same temperature, e.g., the Greenberg (1991) 29˚C dataset and the Byrd and Allen (2001) 30˚C dataset, data from the current study aligns more so with the results obtained in Greenberg (1991). Though Greenberg's cohorts completed larval development approximately 1.5 d earlier than our larvae, the larval development times captured in that study overlap largely with our data. Similarly, the ranges we produced for both larval and pupal development times overlapped with those found in Byrd and Allen (2001). However, we observed post-feeding larvae more than a day earlier, and larval stages were completed more than nine days earlier in our study compared to the maximum larval development times obtained in Byrd and Allen (2001). Furthermore, Byrd's pupal stage at this temperature lasted up to three days longer than those reported in this study. Overall, the data generated in the current study, as well as Greenberg (1991), exhibit a more limited range of developmental diversity compared to Byrd and Allen (2001). Because the overall rearing container is not described in detail in Byrd and Allen (2001) (or in Greenberg (1991)), we cannot use rearing container size to explain the discrepancies between these studies. Greenberg (1991) provides negligible information about the geographic population used in his study, therefore comparisons of our flies to his are limited. However, as Byrd's specimens were collected in Florida and ours were collected in Tennessee, ecoregion differences could have induced a phenotypically plastic effect of the *P. regina* populations in question (38).

An additional explanation of the quicker larval development times in our study compared to Byrd's may lie in interspecific competition of blow fly populations in the areas in which the study organisms originated. The founding *P. regina* used for laboratory colonies in the current study originated from the ARF, which can host 100 – 200 sets of human remains at various stages of decomposition at any given time. One of the most prolific blow fly species that co-occurs at the ARF is the hairy maggot blow fly, *Chrysomya rufifacies* (Macquart) (39), a facultative predator known for its consumptive (16) and non-consumptive effects (40, 41). Such behavior is hypothesized to either drastically reduce competitor (e.g., the secondary screwworm *Cochliomyia macellaria*) populations (16), or to illicit selection for faster development in these competitors in areas where *C. rufifacies* has extended its distribution (42). The area from which Byrd collected his *P. regina* specimens

was likely quite different in terms of resource availability when compared to the ARF. The ARF has provided near-constant resources for *C. rufifacies* for over twenty years (39, 43), likely facilitating a large population of this species and potentially governing the biological responses of interspecific blow flies that co-occur in the area. Although the arthropod community structure of vertebrate remains in the ARF is similar to necrophagous communities at other local sites (43, 44), there has been no investigation into whether the saturation hypothesis holds true between geographically distant ecoregions. Furthermore, though Byrd's *P. regina* specimens were collected at a time when *C. rufifacies* was already established and prevalent in Florida (5), partitioning of carcass colonization by size and season may have prevented the selection of Florida *P. regina* towards quicker development times (45). We hypothesize that the near-constant presence of *C. rufifacies* at the ARF has contributed to the selection for rapid larval development times in *P. regina* (and potentially other early colonizing species) in this area and that these interspecific effects at least partly explain the differences between the current study and Byrd and Allen (2001).

The reference dataset an entomologist uses to estimate larval age in a forensic investigation may not represent the developmental diversity of the local blow fly population in question. As reference datasets are typically derived from flies collected in the geographic vicinity of the author's institution, the data obtained from these flies may be dissimilar to conspecific flies in another geographic region (e.g., our study compared to Byrd and Allen (2001)). The best-case result in such a scenario would be the generation of a different, yet still accurate TOC, whereas the worst-case result would be drastically different and inaccurate TOC estimations for a given set of remains. TOC accuracy assessments of published, commonly used datasets for casework in the US are greatly needed in our field. Such assessments are not meant to diminish the usefulness or credibility of these datasets, but rather to highlight the potential for error under certain conditions.

Our study represents one of the only attempts made in forensic entomology to immediately validate a developmental dataset in the field with human remains. Additionally, the TOC estimations were made possible by knowing when each human donor was initially colonized by blow flies. Though there is a large amount of published, laboratory-derived biological data available for use by forensic entomologists, few studies have ever attempted to validate their work (20, 25-29). Furthermore, validation of thermal summation models under realistic field conditions using human remains is severely lacking given the rarity of human decomposition facilities worldwide. However, such validation attempts are critically important for the field of forensic entomology as they form the basis of reliability and credibility of the science used in medicolegal casework.

Although the results obtained in this study are promising, several limitations must be addressed. First, we have only generated data from a single trial performed at a single temperature. To determine if the trends observed here are robust, further examination of rearing container size on development rate will need to be conducted by increasing replication and trial repetition. This will allow for a stronger statistical association to be made between the biological parameters of interest and the experimental treatments. The addition of more temperatures will allow us to generate a more forensically relevant dataset that is applicable under various environmental conditions. Additionally, though we aimed to capture a robust dataset that included information about overall larval and pupal development rates, the inclusion of a more high-resolution sampling scheme could reveal important differences that were not captured here. Improving sampling from 24-hour intervals to 12- or 8- hour intervals would generate useful data for TOC validation as well as for real-world scenarios in which younger larvae are collected from remains. Finally, future work should include parameters to control for air flow within each of the different sized containers to increase consistency across replicates.

Though there were limitations to the current study, validating the data obtained thus far is an endeavor that has not been regularly attempted in our field, and the ability to use human remains is invaluable in this line

of work. The results from this study align with recent exhortations from the forensic entomology community to properly use insect evidence (46, 47). Transparency regarding the validity of the estimation being made is crucial for strengthening the science and legitimizing the reliability of forensic entomology in the courtroom.

Author Contributions

CGO conceptualized this project, as well as conducted experiments, collected data, collected field samples, analyzed data, and wrote the manuscript. MSM, MES, and RKW helped conduct the experiment, collected data, and wrote the manuscript. HSMZ collected field samples and wrote the manuscript.

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