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RESEARCH ARTICLE

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Forensic analysis of *Chrysomya megacephala* growth patterns for precise post-mortem interval estimation

[1]Fawad Khan, [2]Samina Yasmin, [1]Farman Ali, [3]Gul zamin Khan, [1]Inam Ullah, [4]Bahar Uddin and [5]HA El-Sadawy

[1]Department of Entomology, Abdul Wali Khan University, Mardan, Pakistan.

[2] Department of Zoology, Hazara University, Mansehra, Pakistan.

[3] Chief Scientist, Nuclear Institute for Food and Agriculture, Peshawar, Pakistan.

[4]Department of Zoology, Shaheed Benazir University, Upper Dir.

[5]Department of Parasitology and Animal Diseases, Veterinary Institute, National Research Centre, Dokki, Egypt.

[1]Corresponding Author: Fawad Khan medicalentomologist94@gmail.com

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ABSTRACT

The study investigates growth dynamics at critical stages of decomposition by *Chrysomya megacephala* - Fresh, Bolting, Decay, Actively Decay, and Dry. Meticulous variation in weight under environmental conditions during each stage could reflect distinct growth patterns influenced by temperature, humidity, and availability of substrates to improve the forensic accuracy of PMI estimation. Early larval stages such as Fresh and Bolting recorded moderate weight gain, but the peak feeding activity occurred at the Decay and Actively Decay stages where the weights stabilized due to decreased tissue availability. The existence of high weights during favorable conditions, especially a fresh weight of 10.485 mm on D13, emphasizes how environmental factors promote the rapid growth of larvae, particularly in nutrient-rich environments. Findings show that larvae of *Chrysomya megacephala* are sensitive to environmental changes, thus making their growth patterns very useful in forensic practices. In fact, this study further underpins the species as a strong candidate for being a useful indicator of PMI; because the growth stages tracked against specific conditions allow forensic investigators to make more precise calculations of PMI, presenting a more refined tool in the criminal investigation process.

keywords: Forensic Entomology, *Chrysomya megacephala*, Post-Mortem Interval (PMI), Decomposition Stages

.Introduction

Chrysomya megacephala, also known as the Oriental latrine fly, is a species with a global distribution. These *synanthropic* flies are rather inclined to live close to human habitations and thus have a potential mechanical role as carriers of various pathogens that can indirectly impact human health substantially (Chaiwong et al., 2014). *The Chrysomya megacephala* was the most dominant across all categories of land usage, which clearly showed marked female predominance in both sexes. The populations of this fly could be collected practically throughout the entire year although the numbers tended to increase during late summer months to indicate a favorable association of temperature but an adverse association with relative humidity. The projected abundance of *Chrysomya megacephala* varies across land utilization categories ranging from lowlands to wooded regions (Klan et al., 2011).

Chrysomya megacephala, being a globally important species of blow flies in medical and forensic issues, shows daily flight patterns and seasonal distribution influenced by several environmental factors. The highest abundance was recorded during hot weather, while lower numbers were during the rainy season and winter (Sontigun et al., 2018). The Oriental latrine *chrysomya megacephala*, *Chrysomya megacephala*, is an Asian genus found with an imposing status throughout Asian countries that host diverse distributional patterns on this continent. However though born in a foreign habitat. *Chrysomya megacephala* has created some habitats in Pakistan; consequently, it also supports a form of local biodiversity along these ecosystems (Sebastião and Castro, 2019).

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Chrysomya megacephala, holds a very great importance in various fields of work, such as public health, food industries, medical entomology, death investigation, and lately industrial recycling of organic waste. This usually results in litigation involving forensic entomology. *Chrysomya megacephala* lives in human habitation, adults frequent moist food, decaying carrion, and feces, making them prolific breeders. Added to this, the synanthropic species has spread worldwide beyond its native range. Because it has wide and global consequences of human-related issues connected with *Chrysomya megacephala* (Badenhorst and Villet, 2018). The time for carrion flies to pass through their developmental stages up to the emergence of maggots from carcasses serves as a very important tool for estimating the post-mortem interval in criminal cases. *Chrysomya megacephala* is an important blow fly species in forensic and medical aspects (Bansode et al., 2016). Adult flies are the first insects to colonize a dead body and can lay eggs as early as within a few hours after landing on a cadaver. Therefore, knowing the different stages of development of the larvae of flies is very important for the identification of the species on a dead body, which can assist in forensic investigation in the field (Mendonca et al., 2012).

Materials and methods

3.1 Study Site – Peshawar

The study was conducted in District Peshawar, Pakistan, these locations, at geographical coordinates 34° 00' 28.80" N, and 71° 34' 42.56" E, provided a range of different conditions of urban, suburban, and rural environments that were conducive to studying *Chrysomya megacephala*.

3.2 Blow Fly Collection for Distribution Study

A thorough survey on the distribution of *Chrysomya megacephala* in Peshawar was carried out by sampling different environments with standardized traps, such as modified CDC traps and sticky traps, every week. Long-term sampling ensured that seasonal fluctuations were captured and data included species composition, abundance, as well as accurate coordinates, along with environmental variables such as temperature, humidity, and vegetation cover at each location. Statistical analysis helped reveal the distribution patterns, and GIS software was then used to map them for visualization of the spatial dynamics of blow flies in Peshawar. The study contributes to the forensic and ecological understanding of blowfly populations in that region.

3.3. Sampling Techniques and Preservation

Adult and larval blow flies were collected by sweep nets, forceps, and spoons that were applied across sites in District Peshawar. Adult specimens were placed into labeled plastic bags and kept in freezers to keep the live specimens preserved. Preserved specimens were kept within naphthalene in case the specimens were dead so that proper species identification was done. The preservation and storage method helped in keeping specimens for species identification and later analysis.

3.4 Adult Blow Flies Identification

Adult blow flies were determined by characteristic morphological features on abdominal patterns, body and thoracic spiracle color, eye facet patterns, venation of the wings, proepisternal setae, and head vertical setae. By dichotomous keys, which constitute checks using observable characteristics in classification, scientists identified the species of blow flies successfully. Such identification resulted in a comprehensive understanding of the diversity and distribution of blowflies found in Peshawar.

3.5 Larval Stages: Determination and Rearing

Hot water killing was followed by the preservation of larval specimens in 70% ethanol, which was then maintained in jars containing meat in controlled conditions to allow the emergence of adults. Observation occurred from 10 am to 5 pm every day. The emerged adults were preserved in ethanol for morphological identification; hence, the life stages were well studied, which provided a good species profile for Peshawar.

3.6 Data Recording and Statistical Analysis

Species identity, sex, developmental stage, and corresponding environmental conditions were documented in detail regarding data management. The data were aggregated, and behavior of relationships between blow fly dispersion, environmental parameters, and developmental activities was unraveled with the help of software like Prism, R, and SPSS. This competent statistical approach allowed for the proper interpretation of *Chrysomya megacephala* patterns across habitats.

3.7 Forensic Importance Assessment

The study examines the larval developmental timeline of *Chrysomya megacephala* under controlled environmental conditions focusing on growth stages, temperature effects of the environment, and adult emergence studies. Main forensic indicators included Post-Mortem Interval (PMI).

Results

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The growth rates of *Chrysomya megacephala* larvae differed across the five stages of decomposition: Fresh, Bolting, Decay, Actively Decay, and Dry. Measurements revealed significant fluctuations due to environmental conditions. Low weight gains were recorded at the early stages, including Fresh and Bolting at approximately 0.1 to 0.2 mm, which is consistent with the initial feeding behavior of younger larvae. For instance, the weight measured at the Fresh stage like 0.1055 mm on Day 1 indicates feeding behavior in the early instar whereas it increases but remains in the low-to-moderate range at the Bolting stage, for example, 0.3555 mm on D5, which shows larval growth accelerates once nutrient uptake continues. The weights, however, fluctuated during the Decay stage recorded as high on some days while very low on other days.

It was quite highly possible that such a broad range of weight was due to the amount of tissue decomposing and also that favorable environmental conditions may determine it. Once the larvae entered the Actively Decay stage, they would stabilize their weight on 3.7-4.06 mm indicating maximal decomposition activity where larvae were consuming maximally. The Dry stage also exhibited consistency in consistency with average weight ranging between 3.7 to 3.97 mm; this depicts lower availability of tissue at nearly full decomposition. Moreover, notable outliers in the form of fresh weights of 10.485 mm on D13 show that positive conditions like temperature or surges in humidity significantly influence larval size. The impact is more pronounced during earlier developmental stages. These results underscore the fact that the growth of *Chrysomya megacephala* larvae is highly sensitive to external factors, validating its application in forensic entomology for estimating post-mortem intervals, given the predictable pattern of growth under varying environmental conditions allows for more accurate PMI estimations

Table 1 Growth and Decomposition Stages of *Chrysomya megacephala* Larvae

Days	Weight in mm - Fresh	Weight in mm - Bolting	Weight in mm - Decay	Weight in mm - Actively Decay	Weight in mm - Dry
DAY 1	0.1055	0.1585	1.3192	3.67	3.97
D2	0.125	0.13	0.7088	3.865	3.965
D4	0.108	0.0945	0.2566	3.7	3.625
D5	0.1555	0.3555	3.415	3.7	
D6	0.1185	0.1435	0.5169	3.9	3.69
D7	0.1055	0.1585	1.3192	3.67	3.97
D8	0.1	1.6527	3.665	3.78	
D9	0.11	0.093	0.429	3.92	3.96
D10	0.1	0.0975	0.4335	4.06	3.825
D11	0.12	8.4435	3.89	3.905	
D12	0.1295	0.1265	0.3216	3.835	3.92
D13	10.485	6.375	0.234	3.7	3.865
D14	8.9375	3.4375	0.4318	3.69	3.7
D15	8.75	4.5	0.3696	3.97	3.415
D16	6.25	6.25	1.582	3.78	3.9
D17	4.0625	6	1.5735	3.415	3.7
D20	3.875	8.8125	0.5223	3.665	3.78
D21	3.125	8.05875	9.0767	3.92	3.96
D22	10.5		0.5194		
D23	0.108	0.0945	0.2566	3.7	3.625
D24	0.114	0.1555	0.3555	3.415	3.7
D25	0.1435	0.5169	3.9	3.69	
D26	0.1055	0.1585	1.3192	3.67	3.97
D27	0.1265	0.1	1.6527	3.665	3.78
D28	0.11	0.093	0.429	3.92	3.96
D30	0.1	0.0975	0.4335	4.06	3.825
D31	0.18	0.12	8.4435	3.89	3.905
D32	0.1265	0.3216	3.835	3.92	
D33	10.485	6.375	0.234	3.7	3.865
D34	8.9375	3.4375	0.4318	3.69	3.7
D35	8.75	4.5	0.3696	3.97	3.415

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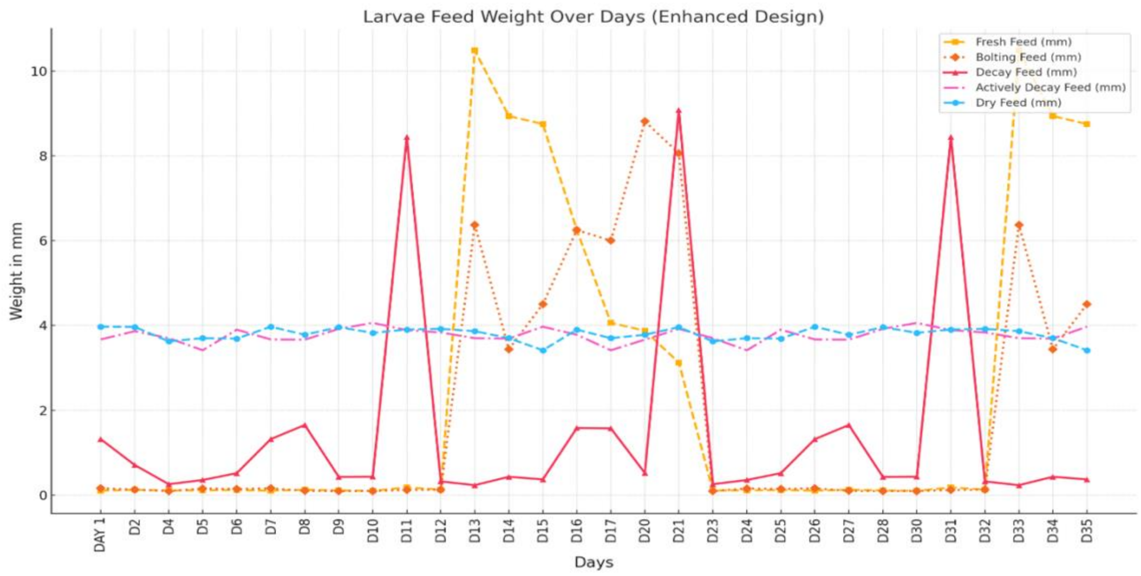

Discussion

This dataset outlines the measurements of the larval weight of *Chrysomya megacephala* at different feeding stages, which include Fresh, Bolting, Decay, Actively Decay, and Dry for several days. All these stages and measurements are critical in determining the extent how which environmental factors impact the decomposition process. The PMI in forensic entomology relies much on these aspects and, hence very critical. Let's proceed and discuss each one in detail to understand the trends and implications:

1.1.1 1. Fresh Weight Stage

Observation: The fresh stage is characterized by low values for weight measurements (0.1 - 0.18 mm), especially in the early days. For instance, on Day 1, the fresh weight is 0.1055 mm corresponding to normal early-stage larval feeding.

Interpretation: This is the first developmental stage during which the larvae begin to feed on the soft tissues. The weights at this stage are very low as they occur during the instars where the main objective of the larvae is locating the feeding sites rather than increasing in weight.

Environmental Impact: Temperature and humidity impact this stage by accelerating or retarding early larval development. High temperatures, as observed on D13 (10.485 mm fresh weight), cause faster tissue breakdown and thus higher larval consumption.

Bolting Weight Stage

Observation: The Bolting stage weight is very variable with values ranging from about 0.093 mm (D9) to as high as 8.8125 mm (D20).

Explanation: This is an active feeding stage where larvae move on to a more intensive feeding phase with higher nutrient consumption. The weights are varying and show that the environment was either changing or substrate supply was variable, thereby causing different growth rates.

Environmental Implication: Bolting weights of D20 and above signify an ideal environment that encourages maximum feeding. This could be associated with both increased temperature and humidity, which facilitate favorable decomposition.

Decay Weight Stage

Observation: Decay weights are highly variable, ranging from 0.234 mm (D13) to 9.0767 mm (D21).

Interpretation: The Decay stage is the peak of feeding activity where larvae are actively decomposing tissue. Higher decay weights indicate that the larvae are in good condition and can take in high amounts of nutrients. Lower decay weights may indicate that the larvae are experiencing environmental stress or that there is less tissue available.

Environmental Impact: The higher the metabolic rate in the larvae, the greater the weight of decay with the increased temperatures. For instance, an extreme weight of 9.0767 mm on D21 might be associated with conditions favorable to environmental factors such as humidity and temperature that can lead to increased

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growth. Lesser decay weights could suggest unfavorable conditions or competition that in forensic applications may turn out to be crucial in making a proper evaluation of PMI.

Actively Decay Weight Stage

Observation: Generally, the actively decay weights stabilize between 3.415 mm and 4.06 mm. The stage is characterized by the steady increase in the larval weight day after day which shows the larva feeds steadily.

Interpretation: This stage represents the maximum tissue breakdown period. The fact that the active decay weights are stable implies there is a stable environmental condition favoring this stage of decomposition.

Environmental Impact: This stage is significantly affected by environmental factors, such as temperature. For instance, on D10 (4.06 mm), weight peaks in this stage when there are favorable temperatures to promote high metabolism rates. Humidity also plays a significant role in this stage because the efficiency of feeding is impacted by too much or too little moisture.

Dry Weight Stage

Observation: In the Dry stage, weights are relatively stable but slightly variable with values ranging between 3.415 mm and 3.97 mm.

Interpretation: This is the final stage of larval development before pupation where the larvae feed on the available remaining nutrients. The consistency in weights day after day implies that this stage has a minimal effect on environmental conditions since the decomposition material has a lesser influence from the outside conditions.

Environmental Sensitivity: The dry weight stage could be less responsive to environmental fluctuations in that the larvae have gained an age where they could tolerate marginally unfavorable conditions. On some days, lower weights indicate adverse humidity and temperature to the last stages of feeding.

General Observations and Patterns

Environmental Influence: The data shows that temperature and humidity play a significant role at all stages, where elevated levels promote faster growth and higher weights. Days like D13 and D21, where weights reach extreme values in specific stages, correlate with favorable conditions, thus speeding up the decomposition process.

Stage-Specific Development: There are different weight ranges corresponding to each feeding stage from the early stages to the later feeding stages. Fresh and bolting have lower weights which can be said to correspond to an initial feeding stage and decay and active decay correspond to higher weight ranges since there is a higher activity of feeding. Dry has stabilized and has marked signs of the end of active decomposition.

Forensic Importance: Such observations are important in forensic entomology, and it is possible to estimate PMI using the developmental stages of *Chrysomya megacephala* larvae. A correlation between weight changes from the different developmental stages allows the forensic expert to make some environmental linkages associated with the observed larval development at the crime scene for more accurate PMI.

Conclusion

Chrysomya megacephala larval development with this detailed information helps depict the influence of environmental conditions on the decomposition process. Distinctive weight profiles can be found in every stage, and the growth rates are highly dependent on both temperature and humidity. Such features are critical for forensic use, especially in PMI estimation, since the developmental stages of larvae and weight profiles can indicate the period of decomposition. Further studies would complement these findings by including other environmental factors such as substrate type or interspecies competition as well, to further develop forensic methodologies and better estimate PMI at different scenes.

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