H21 The Survival of *Calliphora Vicina* (R.-D.) (Diptera: Calliphoridae), a Forensically Significant Blow Fly, Following Submergence Depends on Life Stage and Submergence Time

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Learning Overview: The goal of this presentation is to provide attendees with an overview of blow fly submergence and its importance in forensic entomology.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by highlighting the potential importance of blow flies in aquatic death investigations.

Introduction: Forensically significant blow flies (Diptera: Calliphoridae) are commonly used to estimate the post-colonization interval, which can be used to infer the minimum elapsed time since death.[1] In order for forensic entomologists to produce such estimations, they must first estimate the age of the blow fly specimen collected from the field, using existing reference datasets on the average size and developmental progress of the same blow flies species.[2] These reference datasets are often produced locally in a controlled laboratory setting under different conditions commonly experienced in terrestrial environments, while aquatic factors are often not assessed or reported.[2,3] To address this gap in knowledge, this study examined the impact of submergence on the survival of two immature stages of *Calliphora vicina* (R.-D.) (Diptera: Calliphoridae). It was hypothesized that survival rates, based on successful eclosion, will decrease with increasing submergence time for both third instar post-feeding larvae as well as larvae.

Methods: A total of 1494 post-feeding third instar *C. vicina* larvae and 1,502 pupae (n = 2,996 total samples) were randomly assigned into control and treatment groups of 30 +/- 2 samples each. The larvae treatment groups were submerged for 1-, 2-, 3-, 4-, or 5-hour time intervals in glass jars with 50mL of distilled water, then transferred to another container of saw dust so they would have adequate conditions to pupariate. The controls were not submerged, and were instead transferred directly to a container of saw dust. Once all the larvae pupariated, they were transferred to a petri dish with filter paper. Similarly, the control pupae were transferred directly to a petri dish with filter paper, whereas the treatment groups were submerged for 24-, 48-, 72-, 96-, or 120-hour time intervals in a weighted cheesecloth pouch to ensure that they remained submerged for the full treatment period. They were then transferred to a petri dish with filter paper. Five replicates of all conditions were performed for each time condition, with control groups for each.

Results: Across the 747 larvae specimens assigned to the control group, the mean survival rate at each time period was consistently above the 80% threshold (ranging from an average of 86.0% to a 95.3% survival rate). Larvae in the treatment group revealed a strong negative linear relationship between survivability and hours of submergence. At two hours of submergence, the mean survival was 85.3% but took a drastic decline to 51.7% at three hours. A significant simple linear equation was found ($F(1,1492) = 885.770$, $p < 0.000$), with an R^2 of 0.373. The larval predicted eclosion rate was equal to 0.953 + (-0.151) hours submerged. The control pupae exhibited similar survivability rates above the 80% threshold, but the treatment group starting from the 24 hours of submergence revealed a lower 57.0 % survival rate, which also showed a strong negative linear relationship; a significance regression equation was found ($F(1,1500) = 1629.977$, $p < 0.000$), with an R^2 of 0.521. The pupae's predicted eclosion rate is equal to 0.875 + (-0.008) hours submerged. At 96 hours, the survival rate of pupae reached zero.

Discussion and Conclusion: Rearing specimens to adulthood plays an important role in helping forensic entomologists identify the blow fly species, which is why factors related to the survivability of specimens are important to consider. These findings show a strong negative linear relationship between submergence periods and survivability of *C. vicina* post-feeding third instar larvae and pupae; submergence was found to be a valuable indicator of successful eclosion. These are forensically significant findings because they highlight the importance of collecting specimens quickly when submergence is suspected to reduce the risk of loss of entomological evidence, as well as aiding in submergence interval estimations.

Reference(s):

1. Tomberlin, J.K., Mohr, R., Benbow, M.E., Tarone, A.M., VanLaerhoven, S. A Roadmap for Bridging Basic and Applied Research in Forensic Entomology. *Annu Rev Entomol* 2011; 56:401-421.
2. Tarone, A.M., Sanford, M.R. Is PMI the Hypothesis or Null Hypothesis? *J Med Entomol* 2017; 54:1109-1115.
3. Singh, D., Bala, M. Larval Survival of Two Species of Forensically Important Blowflies (Diptera: Calliphoridae) After Submergence in Water. *Entomol Res* 2011; 41:39-45.

Forensic Entomology, Submergence, *Calliphora Vicina*

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