Effects of Submergence of *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae) Eggs in Water

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Chrysomya rufifacies (Macquart) (Diptera: Calliphoridae) is a medically important blowfly species used to estimate the postmortem interval (PMI) of corpses. *Chrysomya rufifacies* eggs were submerged in water for 1, 3, 5, 7, and 10 minutes to see if the eggs can survive being submerged in water. The number of successfully hatched eggs was recorded and a significant difference was determined between the 10 minutes treatment and the control. Based on the results, *C. rufifacies* eggs can tolerate water until 10 minutes of submergence. This is the first experiment that deals with the drowning of *C. rufifacies* eggs, which is important in determining emergence time of the flies to estimate the PMI of a corpse.

Insects can be a helpful tool to provide forensic scientists with a good estimate of displacement speed, or postmortem interval (PMI). PMI can help scientist provide the age of the larva on a body (Catts, 1992). There are many blowflies that inhabit corpses shortly after death. One prominent species of blowfly to inhabit corpses is *Chrysomya rufifacies* (Sukuntason et al 2008). *Chrysomya rufifacies* is important in PMI determinations because of its highly predictable developmental time and low degree of variation in larval development. The developmental rate of fly larvae depends primarily upon the environment temperature (Sukuntason et al 2008). The oviposition preference of females can directly affect the PMI estimation by delaying or accelerating egg laying (Yang and Shiao 2012). *Chrysomya rufifacies* prefers warm weather and has a short life cycle, is widely distributed geographically, and prefers to colonize large carcasses over small ones. *Chrysomya rufifacies* have a tendency to be found on drier bodies in the field. This experiment was conducted to determine if *C. rufifacies* eggs were able to tolerate low amounts of water. There is no current research to explain this observation or to compare results with. This can aid future studies in understanding how water affects the life cycle of the blowfly, as well as provide more accurate PMI, thus helping scientists in many fields, particularly forensics. Since this research is the first of its kind, more studies need to be done in the future to completely understand how water affects the development of the eggs.

Materials and Methods

Eggs of *Chrysomya rufifacies* were submerged in water for 1 minute, 3 minutes, 5 minutes, 7 minutes, and 10 minutes by wrapping the eggs in a paper towel and

placing the wrapped eggs at the bottom of a plastic cup (Bioquip, Valencia, CA) to keep the eggs underwater. The eggs were then placed in 2 oz plastic cups (Diamond Manufacturing Co, Wyoming, PA) and observed for hatching. The number of successfully hatched eggs was counted and recorded. Each treatment included 35 samples of 10 eggs each and the treatments were replicated three times. Controls were eggs that were not submerged in water, and were placed directly into the cups and observed for hatching. A negative control froze the eggs to make sure that the eggs could actually be killed. The resulting data were analyzed using a one-way ANOVA in SPSS.

Results

There were three replications per treatment. No significant difference among each replication was noted ($p<0.05$). The average total number of successfully hatched *Chrysomya rufifacies* for each treatment was 9 individuals hatched (Figure 1). There was a significant difference between the control and 10 minutes ($M=0.035$; $SD=0.016$). There was no significant difference between the control, 1 minute ($M=0.010$; $SD=0.016$), 3 minutes ($M=0.000$; $SD=0.016$), 5 minutes ($M=0.011$; $SD=0.016$), and 7 minutes ($M=-0.004$; $SD=0.016$) ($p<0.05$). There was a significant difference between the treatment of 10 minutes and 3 minutes and treatment of 10 minutes and 7 minutes (Table 1). The treatment of 10 minutes had fewer successfully hatched *C. rufifacies* than the control, 3 minutes and 7 minutes treatments. All the p-values are of significance proving that even a little bit of soaking delays the hatching process.

Discussion

Understanding the life cycle of the blowfly is an important for forensic entomology (Amendt, 2004). Determination of PMI is an important forensic technique that uses the hatching time of the larvae of certain blowflies to estimate the time of death of an individual (Amendt, 2004). The eggs with any kind of soaking showed a change in the hatching process, thus showing that in more humid areas, a different timeline for the life cycle of the blowfly should be considered. The data shows that *Chrysomya rufifacies* eggs can tolerate water. However, the data shows that the eggs that were submerged for 10 minutes caused a decrease in the number of eggs hatched. These results indicate that, though *C. rufifacies* eggs can tolerate water, their emergence can be affected by water after 10 minutes of submergence. The emergence time of *C. rufifacies* affects the PMI. Since water submergence affects the emergence of *C. rufifacies*, then the PMI of a corpse can be affected. Since this is the first experiment that deals with drowning the eggs of *C. rufifacies*, this research is a good starting point for any further research related to it. Research has been completed on a comparison of temperatures and competition with oviposition and egg survival. Future research can focus on if there is a difference in the time it takes for the eggs to hatch after submergence of the eggs. The importance of timing is more crucial than the effect of drowning the eggs because the PMI estimate is based on timing. If the drowning affects timing in any way, then the PMI could be affected. More studies should be conducted to fully understand the effect of water on the development of the eggs. An understanding of how soaking affects the eggs of blowflies can provide further use for blowflies in forensic entomology in relation to cases

where a body was in a wet enviornment prior to death.

References

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Figures

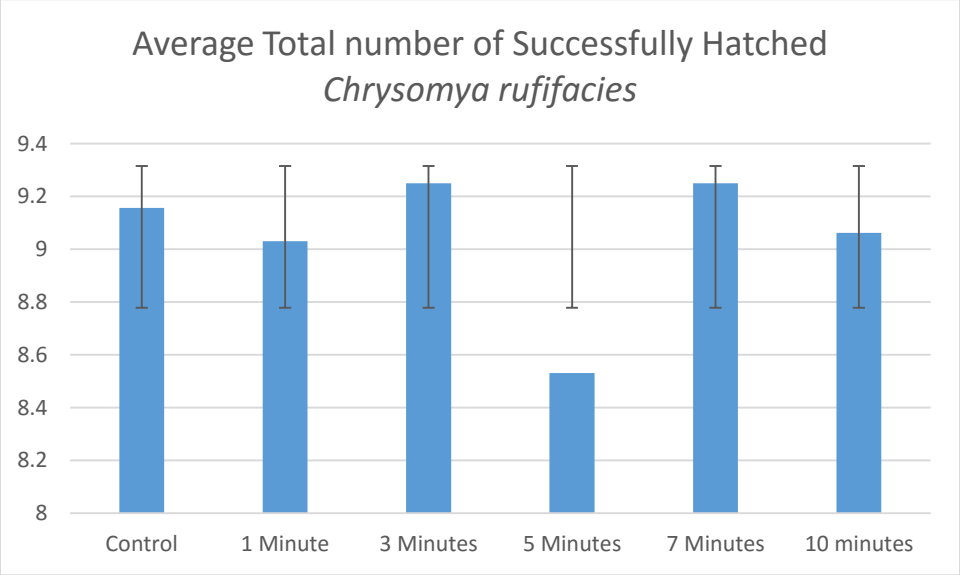

Figure 1. The average total number of successfully hatched Chrysomya rufifacies for each treatment with standard deviation.

Treatment	p-value
1 minute vs. control	0.529
1 minute vs. 3 minutes	0.496
1 minute vs. 5 minutes	0.933
1 minute vs. 7 minutes	0.386
1 minute vs. 10 minutes	0.103
3 minutes vs. control	0.96
3 minutes vs. 5 minutes	0.446
3 minutes vs. 7 minutes	0.85
3 minutes vs. 10 minutes	0.021
5 minutes vs. control	0.478
5 minutes vs. 7 minutes	0.344
5 minutes vs. 10 minutes	0.124
7 minutes vs. control	0.811
7 minutes vs. 10 minutes	0.013
10 minutes vs. control	0.024

Table 1. The p-values for each treatment comparison.