Effect of Water Submersion on *Cochliomyia macellaria* (Fabricius) (Diptera: Calliphoridae) Eggs

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Abstract: *Cochliomyia macellaria* (Fabricius) (Diptera: Calliphoridae) is a significant species in forensic entomology when accurately determining time of death by using postmortem interval. The larvae participate in secondary myiasis when feeding on the decaying tissue of deceased organisms. Variables such as temperature and location can either speed up or slow down the development of *C. macellaria,* which need to be taken into account when determining an accurate time of death. Eggs of *C. macellaria* were collected, submerged in water for varying amounts of time, and observed in order to see if exposure to water caused them to die before hatching. It was found that eggs soaked for one minute had the highest percentage survive. Eggs soaked for zero, two, and five minutes had slightly less survive, while eggs soaked for seven and ten minutes saw a significant decrease in survival. These results will not only assist forensic entomologists when determining time of death, but will also give a better understanding about which habitats this species is most likely to be found in.

Keywords: *Cochliomyia macellaria*, submersion, hatch, post mortem interval

Cochliomyia macellaria is a highly influential species in the fields of forensic and veterinary entomology. Like the remaining three species in their genus, *C. macellaria* have four stages of life: egg, larva, pupa, and adult (Barbosa 2015). They are most prevalent in warm or temperate regions and can be found colonizing on feces, waste, and other substrates including the necrotic flesh of dead human and animals (Boatright 2009). *Cochliomyia macellaria* are known more commonly as the secondary screwworm because the larvae cause secondary myiasis on the decaying tissue that they feed on (Guimaraes 1999). This trait is what makes the species relevant to forensic entomology. Due to their rapid colonization on the remains following death, *C. macellaria* can be used to determine post mortem interval (PMI). Investigators can accurately estimate time of death and whether or not the remains have been moved from their original location by analyzing what stage of life the secondary screwworm is in (Jennings 2015).

Understanding *C. macellaria* development is also significant to agriculture advancements. They are known to transport the eggs of *Dermatobia hominis* (Linnaeus Jr) (Diptera: Oestridae), which can spread disease to livestock leading to enormous economic losses (Barbosa 2014). However, factors such as location of oviposition and weather conditions have the ability to affect growth of *Cochliomyia macellaria.* Knowing how to deter the growth of these organisms can greatly improve the odds of livestock survival.

In this experiment, eggs of the secondary screwworm are soaked in water from one to ten minutes prior to being allowed to hatch freely can successfully hatch and survive. These results will test *C. macellaria's* ability to thrive in water, and indicate if they would be capable of surviving in natural conditions such as rain or flooding. Knowing how water affects hatching will assist in determining a more precise time of death of the remains the *Cochliomyia macellaria* are collected from. It is also useful when predicting if the species will be prevalent in specific environments based on their typical precipitation patterns.

Materials and Methods

Eggs from *C. macellaria* were collected from the Texas A&M University F.L.I.E.S. facility. A control set of 35 groups containing ten eggs each were placed in plastic cups (Reditainer, Port Richey, Florida) and observed hourly for hatching. Another set of 35 groups, each still containing ten eggs, was prepared. This time they were all submerged in distilled water for one minute, then placed by group in plastic cups and observed for hatching. This process was repeated for four additional submersion treatment times: three, five, seven, and ten minutes. Following submersion, the number of eggs in each group of ten were observed hourly. Each treatment time was repeated a total of three trials.

Results

The eggs submerged in water for one minute had the highest hatching rate at 96.56%, followed by the eggs submerged for three minutes at 96.46%. Out of the control group, 96.14% were observed to hatch and 96.08% of the eggs soaked for five minutes hatched. The seven and ten minute submersion treatments saw a significant drop of approximately 3-4%. Only 92.71% of the eggs soaked for seven minutes hatched, while 92.24% of the eggs soaked for the full ten minutes hatched, making that the lowest hatching rate. The averages of each of the three trials for all treatment times, as well as the overall mean of each time, can be found on Table 1 below. The differences between the means of each treatment are discussed in Table 2. ANOVA sig values are plotted below on Figure 1 as well. The amount of times shown along the bottom are compared with the amounts of time listed to the right. Values of 0.05 or less are considered significant, which are seen on the graph because the bars are significantly shorter than the rest. Eggs treated for seven and ten minutes are significantly different from all other treatment times except each other, (with a value of 0.482) which is explained by the 3-4% of eggs hatching. This is also supported due to the significantly higher differences among their means and the means of the other treatment times (excluding the difference between each other) found on Table 2.

	Trial 1	**Trial 2**	**Trial 3**	**Mean**
Control Group	92.76 %	96.80%	98.11%	96.14%
1 min submerged	96.12%	97.99%	95.42%	96.56%
3 min submerged	94.47%	97.40%	94.83%	96.46%
5 min submerged	96.36%	98.29%	93.33%	96.08%
7 min submerged	88.25%	95.86%	94.41%	92.71%
10 min submerged	83.34%	96.56%	95.14%	92.24%

Table 1. Hatch rate averages for each trial and overall mean of each treatment time

	Control	1 min	3 min	5 min	7 min	10 min
Control	0	-.0077	.0024	-.0028	.0316	.0415
1 min	.0077	0	.0100	.0049	.0393	.0492
3 min	-.0024	-.0100	0	-.0051	.0292	.0392
5 min	.0028	-.0049	.0051	0	.0344	.0443
7 min	-.0316	-.0393	-.0292	-.0344	0	-.0099
10 min	-.0415	-.0492	-,0392	-.0443	-.0099	0

Table 2. Differences between the overall mean for each treatment time

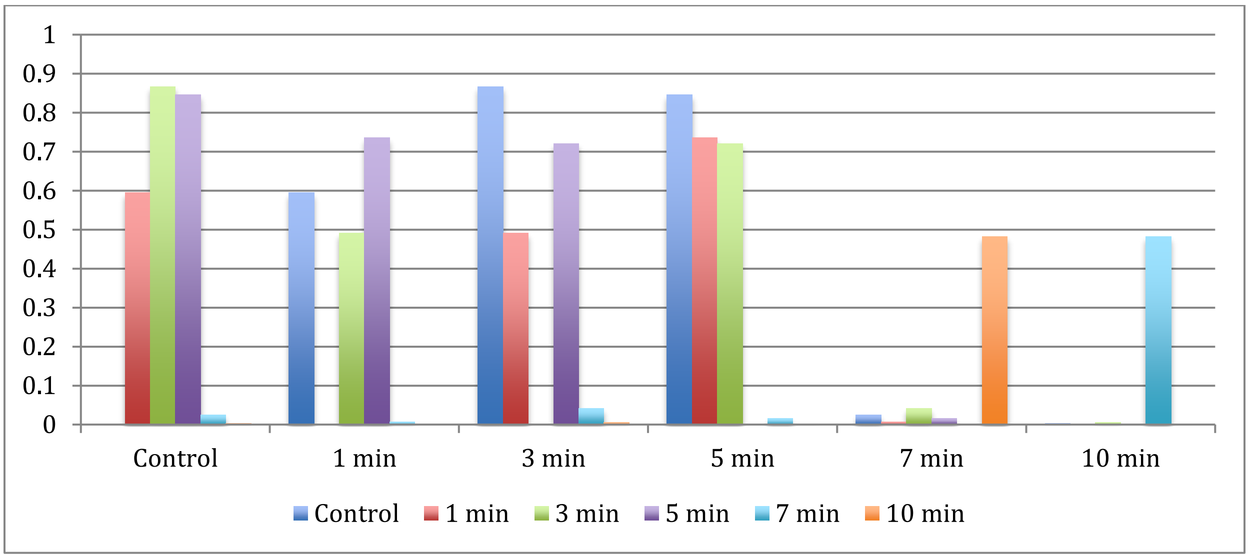

Figure 1. ANOVA sig values

Discussion

Calliphoridae are most prevalent during the warmer seasons of spring and summer because they develop more rapidly in warmer temperatures (Jennings, 2015). This can be seen in Owing's experiment where the growth of *Cochliomyia macellaria* was monitored in three different regions of Texas all with varying climates. The organisms in the warmer regions developed at a much faster pace than that of the organisms in cooler environments (Owings, 2014). Precipitation patterns are an important factor when determining where the secondary screwworm has the best chance of survival as well. Understanding the type of habitat *C. macellaria* thrive in makes is easier to predict in which habitat this species is more likely to be widespread. It is important to know where these organisms reside so precautions for possible disease vectors (such as the spread of *Dermatobia hominis* can be taken. Understanding how eggs and the other stages of *C. macellaria* develop when exposed to water is also a significant factor when determining time of death of the organism on which they were feeding. For example, if larvae were on human remains found outside and knew it had rained the day before, this might change the postmortem interval. Finding the accurate time of death is critical, especially in criminal cases, so all factors, including precipitation, must be taken into account.

The eggs soaked for zero- five minutes all received relatively similar results; each group had approximately 96% of all eggs hatch successfully. Although the eggs soaked for seven- ten minutes did see a significant ($p \leq 0.05$) decrease as demonstrated in the ANOVA, 92% of the eggs successfully hatched which is a significant amount. Therefore, water submersion did not have a substantial impact on the rate at which the eggs hatched. However, in the natural environment where they eggs found, it is highly unlikely that when subjected to water, the exposure would last under ten minutes. Rainfall typically lasts longer than ten minutes, and if the area in which the eggs are laid became flooded it would most likely take a longer time to drain. Water quality and cleanliness are variable in nature as well. Unlike water that is purified and distilled in the lab, water found in the outside environment is likely to contain dirt, germs, and bacteria. There is limited research on how water submersion effects the development of eggs, but performing an expanded version of this experiment could provide a better understanding of how growth is affected. The eggs should be exposed for a prolonged amount of time, or even set up not to soak, but receive water in drop form (like rain) in order to receive more accurate results.

References

Barbosa, R.R. De Carvalho Queiroz, M.M, Mendonca, P.M., and Dos Santos-Mallet, J.S., 2014. Ultrastructure of Immature Stages of *Cochliomyia macellaria* (Diptera: Calliphoridae), a Fly of Medical and Veterinary Importance. Parasitology Research.

Boatright, S.A. and Tomberlin, J., 2009. Effects of Temperature and Tissue Type on the Development of *Cochliomyia macellaria* (Diptera: Calliphoridae). Journal of medical entomology.

Guimaraes, J.H. and Papavero, N.,1999. Myiasis in man and animals in the neotropical region. Bibliographic database.

Jennings, D.E., Hamilton, G.C., Tomberlin, J.K. and Weidner, L.M., 2015. Seasonal and Geographic Variation in Biodiversity of Forensically Important Blow Flies (Diptera: Calliphoridae) in New Jersey, USA. Oxford University Press.

Owings, C., Spiegelman, C., Tarone, A., Tomberlin, J. 2014. Developmental variation among *Cochliomyia macellaria* Fabricius (Diptera: Calliphoridae) populations from three ecoregions of Texas, USA. Springer-Verlag Berlin Heidelberg