Mortality of *Lucilia sericata* (Meigan) (Diptera: Calliphoridae) Eggs after Distilled Water Submergence

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Edited by Marina Conner

Abstract: *Lucilia sericata* (Meigan) (Diptera: Calliphoridae) is an important species both forensically and medically. This species was often found on the low humidity environment of sheep fleece, so it was predicted that it may not be able to tolerate small amounts of water. *Lucilia sericata* eggs were reared and submerged in 10 ml of distilled water for varying amounts of time. The treatments consisted of one, three, five, seven, and ten minutes of submergence. The percentage of eggs hatched ranged from 47.34% to 52.17%. An ANOVA test showed that there was no significance between the percentage of eggs hatched of the one-, three-, seven-, and ten-minute treatments and the control ($p > 0.05$), but that the five-minute treatment was significant compared to all other treatments and the control ($p < 0.001$). The five-minute treatment could be erroneously significant, because the percentage hatched does not appear significant from the other treatments. Due to no significant difference being observed between all other treatments, it was concluded that the water did not have a negative effect on the hatching of the eggs. This study can aid in the improvement of rearing *L. sericata*, because their development was not hindered by exposure to water. The tolerance range of eggs hatched based on the length of time submerged in water could serve as a starting point for the development of new laboratory rearing techniques. Further experimentation could also determine if the water could increase the percentage of eggs that were hatched.

Keywords: Lucilia sericata, submergence, waterproofing, rearing

A large amount of research has been conducted on *Lucilia sericata* (Meigan) (Diptera: Calliphoridae), due to the veterinary, forensic, and medical interest. This fly is of major forensic importance because it is one of the first species to colonize a corpse and its stage of development can be used to determine post-mortem interval (PMI) (Clark et al. 2006). The ability of a gravid female to quickly colonize a corpse is possible through the use of semiochemicals associated with feeding flies (Brodie et al. 2015).

Lucilia sericata is also pest of sheep in the northern hemisphere and some parts of the southern hemisphere (Erzinclioglu 1987). They can have an economic impact on the sheep industry because they are facultative myiasis ectoparasites, making them of veterinary importance (Erzinclioglu 1987). These flies take advantage of open wounds and places with urine and feces on sheep and

deposit their eggs close to those locations (Wall et al. 1992). After emerging, the maggots feed on the sheep's already damaged tissue which can lead to more damage from infection or other opportunistic insects (Wall et al. 1992). The larvae of *L. sericata* is of medical importance due to its use in maggot debridement therapy (Chambers et al. 2003). *L. sericata* maggots can be used to treat diabetic ulcers and other chronic wounds (Chambers et al. 2003). Not only do the maggots feed on the damaged tissue but they secrete chemicals that have healing properties (Chambers et al. 2003).

Though *L. sericata* has been observed to deposit eggs on carrion, they are more often found laying eggs on sheep since they are stronger as ectoparasites of sheep and don't compete as well with other larvae species that are specialized to feed on carrion (Smith and Wall 1997). The sheep fleece provides a dry environment for the eggs because of its low humidity content (Davies 1948). Based off this observation, it was predicted that *L. sericata* eggs could not tolerate even small amounts of water. However, past research has shown that *L. sericata* eggs were equipped to tolerate higher humidity levels (Davies 1948). The outer egg shells of *L. sericata* are mostly comprised of the chorion and chorionic vitelline membrane both of which are proteins (Davies 1948). Before being deposited, these proteins of the outer egg shell are lipidized which results in a rigid but not waterproof egg structure (Davies 1948). Then a waterproof layer is secreted in between the chorion and the chorionic vitelline membrane (Davies 1948).

Since prior studies have focused on humidity levels this experiment focused on *L. sericata* egg's water tolerance to see if exposure to distilled (DI) water would affect their development. their development was measured by the percentage of eggs hatched after varying submergence times in the DI water. The information from this experiment may not only increase knowledge of the species' development, but it could also improve rearing practices. Improved rearing practices could be utilized in all research that would require the laboratory raising of *L. sericata* and would increase the efficiency at which the research could be conducted.

Materials and Methods

De-agglutination.

Colonies of adult *L. sericata* were obtained to generate eggs for use in the experiment. Each colony was kept until a large egg mass had formed. The egg mass was removed from the colony and placed on a paper towel (Kimberly-Clark, Irving, TX) moistened with water. The paper towel was folded over the egg and allowed to sit for five minutes. After five minutes, the egg mass was stirred with a glass rod (Kimble Chase, Rockwood, TN) until the eggs had undergone de-agglutination.

Submergence.

In order to meet the required number of eggs for the experiment, 2450 eggs were used for each replication. The control for the experiment consisted of eggs that were not submerged and allowed to hatch. Ten eggs

each were placed on 245 pieces of squared black cloth (Vileda Professional, Aurora, IL). The cloths were folded into quarters and 35 of each were placed in a beaker (Corning Incorporated, Corning, NY) for five different treatments. The cloths were covered with 10 ml of distilled (DI) water and allowed to sit for the amount of time correlated to each treatment. The treatments were one-minute, three-minute, five-minute, seven-minute, and ten-minute of submergence in the DI water. After the time, the fabric was removed from the beakers and the water was allowed to drain. The fabric with the eggs were placed into 35 plastic cups with lids (Newell Brands, Ogdensberg, NY) per treatment and placed in a 27°C chamber. Each treatment was repeated for three replications. Using SPSS (IBM, Armonk, NY), an ANOVA test was run on the results of the experiment to determine significant differences between different treatments.

Results

Overall, the experiment yielded relatively low hatch rates across the board. The seven-minute treatment had the highest percentage of hatched eggs at 52.17% and the control had the lowest percentage of hatched eggs at 47.34% (Fig. 1). These values were found to have no significant difference ($p=0.788$) (Fig. 1). The one-minute treatment had a hatched egg percentage of 48.41% and the three-minute treatment had a slightly higher percentage of hatched eggs at 48.97% (Fig. 1). The five-minute treatment had a hatched egg percentage of 49.14% and the ten-minute treatment had the second highest percentage of eggs hatched at 49.90% (Fig. 1).

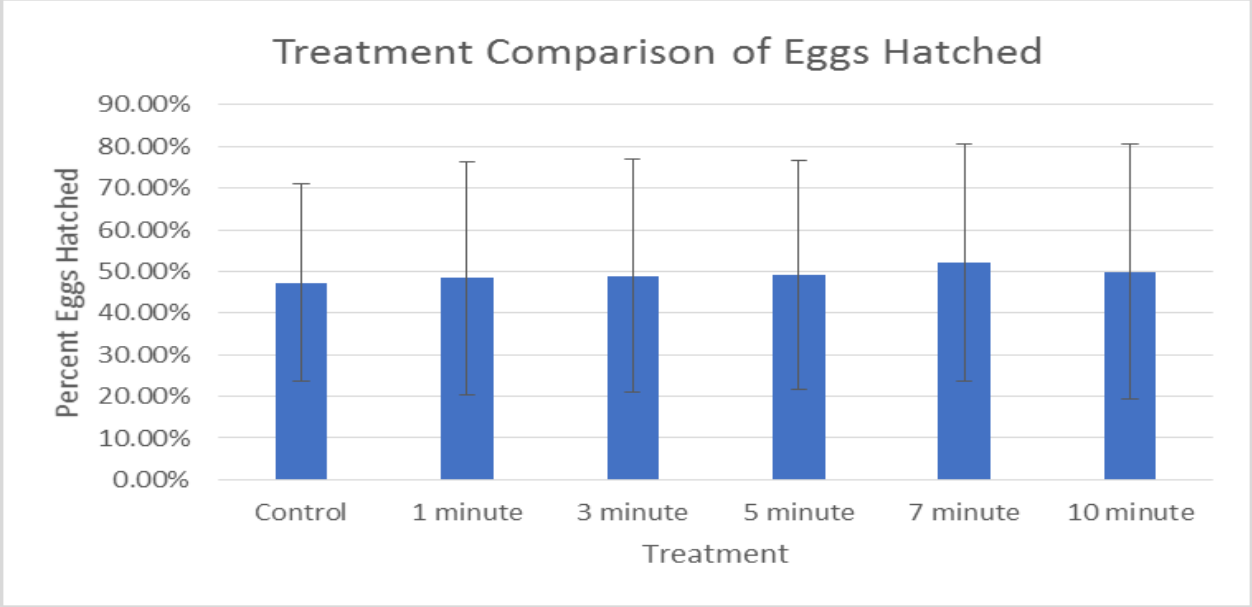

Fig. 1. Mean percentage of *Lucilia sericata* eggs hatched for each DI water treatment, 1-minute, 3-minute, 5-minute, 7-minute, 10-minute and control non-DI water treatment with their respective standard deviations.

Table 1. The Mean, Standard Deviation, Standard Error, and 95% Confidence Interval for the percent *Lucilia sericata* eggs hatched after the Control treatment and the 1-minute, 3-minute, 5-minute, 7-minute and 10-minute DI water treatments respectively.

Treatment	Mean	Standard Deviation	Standard Error	95% Confidence Interval
Control	0.4733786	0.235773612	0.023119505	0.045313397
1-minute	0.4841136	0.279656924	0.027422618	0.053747343
3-minute	0.4897083	0.279656924	0.027422618	0.053747343
5-minute	0.4914125	0.275090906	0.026974883	0.052869799
7-minute	0.5216825	0.283772696	0.027826202	0.054538354
10-minute	0.498972	0.306968099	0.030100699	0.058996285

The values for mean eggs hatched all had relatively high standard deviations with the lowest standard deviation being the control 0.2357 and the highest being ten minutes at 0.3069 (Fig. 1, Table 1). The ten-minute treatment had the highest standard error at 0.0301 and the control had the lowest standard error at 0.0231 (Table 1).

Table 2. Comparison of P-Values with 95% Confidence Interval for the percent of *Lucilia sericata* eggs hatched after each DI water treatment and control treatment using an ANOVA test.

Treatments	Control	1	3	5	7	10
Control		0.940	0.909	0.921	0.788	0.880
1	0.940		0.968	0.929	0.842	0.938
3	0.909	0.968		0.929	0.837	0.969
5	0.000	0.000	0.000		0.000	0.000
7	0.788	0.842	0.873	0.932		0.903
10	0.880	0.938	0.969	0.931	0.903	

Just as the other values, the control had the lowest 95% confidence interval at 0.0453 and the ten-minute treatment had the highest confidence interval at 0.0589 (Table 1). The ANOVA test that was run yielded no significant differences between the one-minute treatment (p=0.940), three-minute treatment (p=0.909), seven-minute treatment (p=0.788), and ten-minute treatment (p=0.880) when compared to the control (Table 2). The five-minute treatment was significantly different from every other treatment ($p < 0.001$) (Table 2). The other treatments had no significant differences present between them (Table 2).

Discussion

No significant differences were found between the one-, three-, seven- and ten-minute treatments the control treatment ($p > 0.05$) (Table 2). Though there was a significant difference of the five-minute treatment from all other treatments based on the ANOVA test ($p < 0.001$) (Table 2). The significance of the five-minute treatment does not align with the data from figure 1

which displays the percentages of eggs hatched based on the treatment all with no treatment standing out as appearing of a significantly higher percentage (Fig. 1). Further experimentation can be done to determine if the five-minute treatment was actually significantly different from the other treatments.

This experiment would indicate that the submersion in DI water did not have any negative effects on the eggs' ability to successfully hatch. The control had the lowest percentage of eggs hatched, 47.34%, which could mean that *L. sericata* eggs benefit from the presence of some water. This supports previous research by Davies & Hobson 1935 when they reared *L. sericata* at 90% humidity for use in myiasis (Davies and Hobson 1935). Further testing is needed in order to determine the significance of the benefit water may have on the eggs. Confirmation of this could explain the relatively low hatch rate of the eggs in this experiment by showing that the eggs could possibly need more water. It was found in a past experiment that high humidity levels did not have a negative effect on L. *sericata* eggs raised in the laboratory, which would further support the conclusion reached from this experiment (Davies 1948). This is most likely due to the waterproofing layer on the surface of the egg (Davies 1948). The information obtained from this experiment could be useful to the forensic field when trying to determine PMI using *L. sericata* (Clark et al. 2006). The larvae are most likely found more often on the dryer environment of the sheep body rather than carrion in the field due to the larval competition from different carrion species (Smith and Wall 1997). The results of this experiment could also aid with the rearing of *L. sericata*, because the eggs do not need to be kept completely dry. Further experimentation could possibly conclude that submerging the eggs in DI water would be beneficial for the rearing process.

This experiment suggests that exposure to DI water did not have any negative effects on the hatching of *L. sericata* eggs. With this knowledge, it can now be tested whether or not exposure to DI water is beneficial to the eggs and to what extent it may be. This information, like the information obtained from many other experiments can be used to better the forensic methods used to rear this species. Future experiments will benefit from the information of this experiment from both the increased rearing efficiency and the improved prior knowledge of the life cycle of *L. sericata* that this experiment provides.

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