

# Toleration of *Lucilia cuprina* (Diptera: Calliphoridae) Eggs to Agitation Within Insect Saline

Christina Kaye Alvarez and Dr. Adrienne Brundage

Editor: Colton Cooper

---

**Abstract:** Microorganisms have an ability to attach themselves to surfaces, where they then multiply and form a protective biofilm, which protects the microorganisms from outside influences, including chemicals for sanitation. This makes the cleaning of fly eggs for maggot therapy difficult. The purpose of this study was to test the toleration of *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae) eggs for agitation, which is used to degrade biofilms. *Lucilia cuprina* eggs were washed with insect saline and treated by undergoing five minutes of agitation. It was found that there was a significant difference between the control group, which did not undergo any agitation, and the treatment group, which did. This knowledge is useful for further testing of the extent to which agitation impacts survivability of the eggs and the debridement of the biofilm.

*Keywords:* *Lucilia cuprina*, maggot, debridement, biofilm

---

## Introduction

Microorganisms have a propensity to attach themselves to surfaces, where they then multiply and often form a protective biofilm (BF) (Chai et al., 2014). These biofilms are made up of many different substances such as proteins, nucleic acids, mineral ions, and other debris from the cell (Orgaz et al., 2006). Biofilms serve as protective environments for bacteria under unfavorable conditions and have drawn attention as serious hygiene problems due to their ability to grow in numerous environments (Hughes et al.). BFs are also highly resistant to degradation and physical agitation, and no extensively applicable and effective technique has been found to deteriorate them without causing adverse

side effects (Chai et al., 2014). This is no different for insect eggs, whose surfaces can be contaminated by various microorganisms and for whom research is still ongoing to determine effective methods of surface sanitation, especially for use in maggot therapy.

Maggot therapy is the deliberate treatment of wounds using fly larvae, and the species *L. cuprina* is useful in this type of treatment (Whitaker et al., 2007). The use of *L. cuprina* for maggot therapy results in the removal of necrotic tissue, the elimination of infecting microorganisms, disinfection of the wound, and stimulation of wound repair (Parnes and Lagan, 2007). While the application of *L. cuprina* maggots are helpful in the healing of chronic wounds,

the eggs that are used for maggot therapy need to be sterilized before they can be utilized in order to avoid further infection. However, sterilization of the egg surfaces can be difficult due to the presence of protective BFs.

BFs must be removed before the eggs are treated with chemicals in order to optimize sterilization. One of the ways to remove a BF is through agitation. This action, while decreasing the amount of biofilm on a surface, may have negative consequences on the eggs themselves. This study is interested in determining the toleration of agitation in *L. cuprina* eggs by observing the hatching rate of agitated eggs versus non-agitated. It is that eggs that undergo agitation will have a lower hatching rate than those that do not.

## Materials and Methods

To start the colony, the maggots of *Lucilia cuprina* were isolated from a carrion body found in College Station, Texas, and they were placed on 50 g of liver (Foster Farms, Hockley, Texas), until they pupated. The pupae were placed in 12x12x12 adult fly cages (Bioquip, Rancho Dominguez, California) and, once they emerged, the pupae were maintained in the colony under a 12:12 L:D cycle. To eat and drink, the flies were given dry sugar (C&H brand, Crockett, California) in a dish and water on cotton balls (Curad, Mundelein, Illinois), *ad libitum*. Two days after eclosion, the adult flies were given 50 g of liver to serve as a protein meal. The eggs were obtained from fed flies by placing 10 g of fresh cow liver in the colony for three hours to serve as an

oviposition site after which the eggs were removed from the liver for deagglutination.

In order to prepare the eggs, they were first removed from the liver using a hobby size 2 paint brush (Prang, Heathrow, Florida) before being placed on a paper towel moistened with RO water. A moist black cloth (Windham Fabrics, Jersey City, New Jersey) was folded over the egg clutches and allowed to sit for approximately five minutes. Then the egg clutches were stirred with the paint brush until broken apart and thoroughly mixed, after which 30 eggs were placed on each 13 mm GE Magna nylon membrane filter (Whatman, Piscataway, New Jersey).

To prepare the cups, nutrient agar was autoclaved and prepared according to directions, and approximately 5 ml was poured into each portion cup. Lids were placed on the cups and the agar was allowed to solidify. Once the eggs were treated, they were removed from the filters using a paint brush and placed on the agar for observation.

The general wash, the wash that all the eggs underwent before respective treatments, was performed first by placing the filters with the eggs into the sterilized filter holder (Andwin Scientific, Schaumburg, Illinois). Then 2 ml of insect saline (Pringle, 1938) was drawn into a sterile 20 ml Leur-lock plastic syringe (Thermo Scientific, Waltham, Massachusetts), which was then attached to the filter holder. The insect saline was forced into the filter holder until the eggs were completely submerged. For the control group, the eggs were then left for five

minutes after which the rest of the saline was forced through the filter, and the eggs were then removed and placed on the nutrient agar in the cups. The treatment followed similar protocol up through submersion. Once the treatment group was submerged, the filter and syringe were attached to an EMD Millipore Rotary Agitator (Merck Millipore, Billerica, Massachusetts) and agitated at 5 rpm for five minutes. Then the remainder of the insect saline was forced through the filter. Lastly the eggs were removed, placed on the nutrient agar in the cups, and observed for hatching.

## Results

The data showed that the hatch rates of the agitated eggs were significantly lower than those of the non-agitated controls ( $p=0.012$ ). The Control group showed a mean hatch rate of 70.46%, with a standard deviation of 0.094793 and a standard error of 0.029976 (Fig. 1). The Treated group had a mean hatch rate of 53.38%, a standard deviation of 0.126802, and a standard error or 0.040098 (Fig. 1).

## Discussion

There was found to be a significant difference between the control and the treatment. The eggs that underwent no

agitation had a higher hatching rate than those that were agitated for 5 minutes. This indicates that the agitation of the eggs inhibited their ability to hatch. Whether it was the action of the agitation itself that killed the insects directly or the cause of the loss of hatching ability by internally damaging the egg is unknown. However, this study depicts the negative effects that agitation can have on maggot therapy eggs. This can then be used in concert with studies on egg survivability in other sanitation methods, such as varying chemical treatments with chemicals like formalin or NaClO or other BF degradation techniques (Thyssen et al., 2013). With a greater understanding of egg hatch rates under various stressors found in sanitation methods, it will allow for further research into more effective approaches.

Further research into egg agitation could be beneficial by measuring the effectiveness of biofilm degradation at different amounts of agitation. Other studies could be performed on estimating how egg hatch rate decreases as agitation times increases. Taking the results of these two potential studies could allow for the analysis of cost and benefit in the use of *L. cuprina* for maggot therapy. For example, if there is an abundance of eggs, then excessive agitation may not be an issue, but if there is a shortage, the amount that is considered an acceptable number of microorganisms on the surface of the eggs must be determined.

Due to the decrease in egg survival, agitation was shown to negatively impact the unhatched insects. This is similar to what has been found previously: that there are

methods for BF degradation, but that many still manifest negative effects on the eggs (Chai et al., 2014). Further studies could elucidate the degree to which agitation

impacts egg survivability, which could be used to make more effective methods of egg sanitation.

### Acknowledgements

I would like to thank Dr. Adrienne Brundage of Texas A&M University for providing me the necessary data and experiment information that this paper is about.

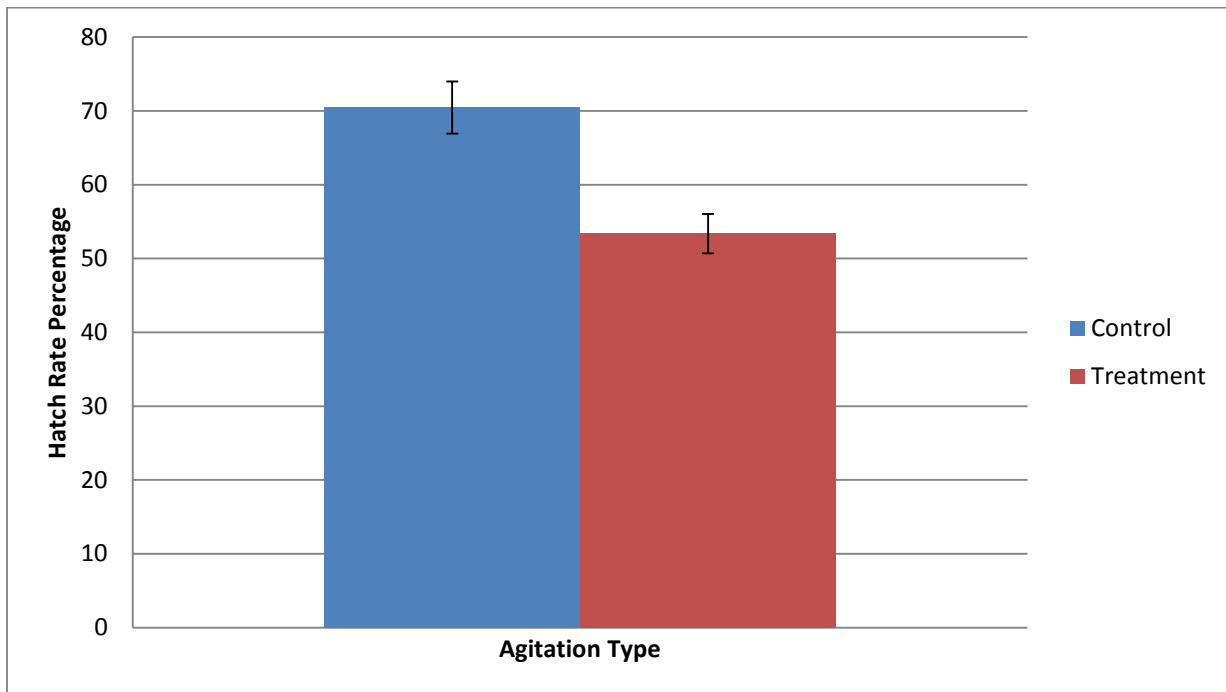


Figure 1. Bar graph comparing the hatch rate percentage of the control group versus the agitation-treated group with the standard error included, showing there was a difference between the two groups.

## References

**Chai, Z., Wang, J., Tao, S., & Mou, H. 2014.** Application of bacteriophage-borne enzyme combined with chlorine dioxide on controlling bacterial biofilm. *Food Science and Technology*, 59(2), 1159-1165.

**Hughes, K.A., Sutherland, I.W., Jones, M.V. 1998.** Biofilm susceptibility to bacteriophage attack: the role of phage-borne polysaccharide depolymerase. *Microbiology*, 144, 3039–3047

**Orgaz, B., Kives, J., Pedregosa, A.M., Monistrol, I.F., Laborda, F., SanJose, C. 2006.** Bacterial biofilm removal using fungal enzymes. *Enzyme and Microbial Technology*, 40, 51–56

**Parnés, A., Lagan, K.M. 2007.** Larval therapy in wound management: a review. *Int. J. Clin. Pract.* 61: 488–493.

**Pringle, J. W. S. 1938.** Proprioception in insects. I. A new type of mechanical receptor from the palps of the cockroach. *J. Exp. Biol.* 15, 101-13.

**Thyssen PJ, Nassu MP, Nitsche MJT, Leite DS. 2013.** Sterilization of immature blowflies (Calliphoridae) for use in larval therapy. *J. Med. Med. Sci.* 4(10):405-409

**Whitaker, I. S., C. Twine, M. J. Whitaker, M. Welck, C. S. Brown, and A. Shandall. 2007.** Larval therapy from antiquity to the present day: mechanisms of action, clinical applications and future potential. *Postgrad. Med. J.* 83: 409–413