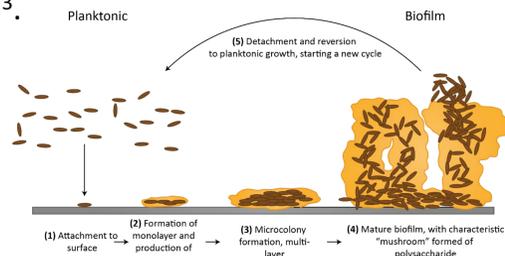


Background

When bacteria adhere to a surface, they produce a biofilm composed of DNA and polysaccharides. Within this biofilm, which one can consider a protective shelter, bacteria can perform immense amplification of gene transfer and quorum sensing. They are also extremely hard to get rid of, which is why biofilms contribute to a lot of medical, veterinary, and agricultural problems. The house fly is a well-known vector that can carry over hundreds of pathogens². It was found that crops in the foregut are an ideal location for bacterial growth in both house flies, fruit flies, and tsetse flies. The crops are essential structures regarding pathogen hospitality and transmission. It has a chitinous cuticular lining in which the bacteria can easily adhere to and it is from the crop that flies regurgitate microbes, along with their saliva. Here, I demonstrated that biofilms can be visualized within the crop of controlled and wild house flies, which has never been done before. I used the bacterium *Serratia marcescens* as a control. *S. marcescens* is a gram-negative, rod-shaped bacteria that produces a red-pigmented biofilm that is easily identifiable and visualized³.



Discussion

Here, I exhibited bacterial growth and proliferation within a host. Most studies of biofilm have been performed in laboratory settings¹, like this one, however, using new techniques, I have visualized biofilm within the crops of wild flies. Biofilm, when visualized, masks clear pictures of bacteria, making them appear blurry, as demonstrated in the results. All work was complete in a laboratory, but it can be applied in medicine, veterinary, and food science settings. This work provides a methodology in which biofilms can be studied in vivo and in a way in which pathogens and bacterium are often exposed to populations. It can be used as an indicator step when processing bacterial infections.

Results



Figure 1: Full, healthy crop and crop duct of *Musca domestica* imaged at 4x. Both lobes are visible as is the musculature that rings around the bilobed structure.



Figure 2: Wild fly crop stained with safranin. Dark spots indicated with black arrows are bacterial colonies. Image magnification 4x.

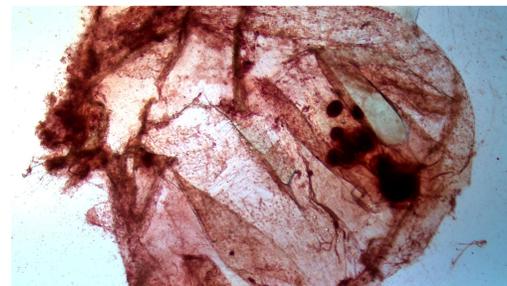


Figure 4: Control crop of *Musca domestica* feed *Serratia marcescens*. Bacterial biofilms indicated with black arrows. 4x Magnification.

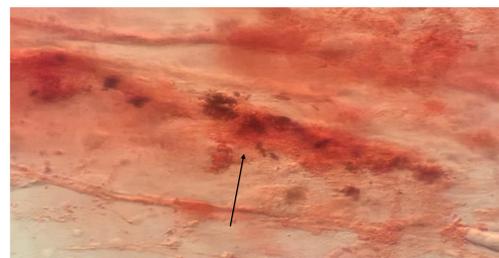


Figure 6: (A) *Musca domestica* crop stained with safranin at x100 magnification with oil immersion. Black arrows indicate bacteria found.

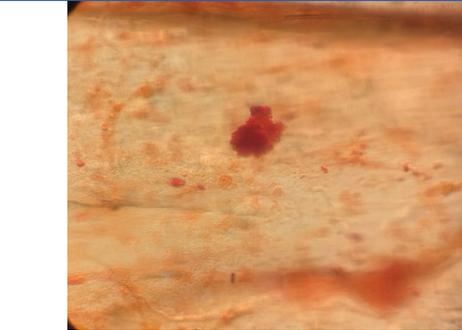


Figure 3: 100x image of one bacterial culture found in the dissected wild fly crop of figure 2.

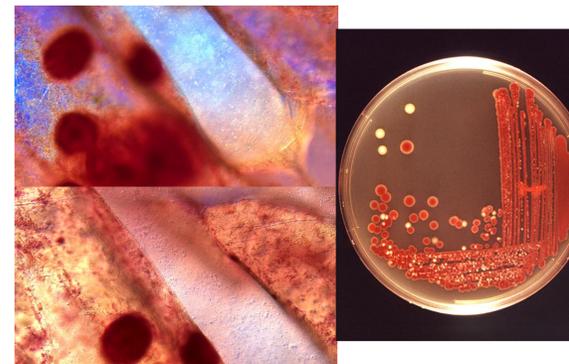


Figure 5: Enlarged view of one bacterial colony found within the *Musca domestica* crop at 100x is on the left. On the right, plated *Serratia marcescens* as a reference to the circular colonies formed.

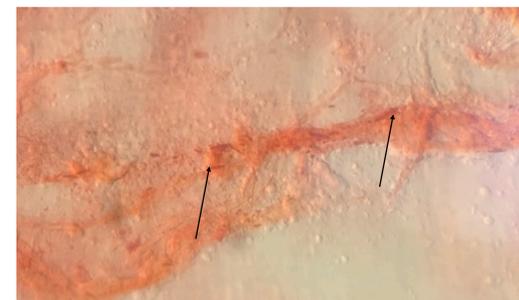


Figure 6: (B) *Musca domestica* crop stained with safranin at 100x magnification with oil immersion. Black arrows indicate bacteria found.

Methods

Bacterial Biofilm

Serratia marcescens was plated + cultured on LB agar. To visualize its biofilm in vivo, a colony was smeared and stained on a clean glass microscope slide.

Bacterial Oral Infections

2-day old flies were starved for 16 hours before being placed in treatment containers in groups of 2-3. A 2cm piece of filter paper was saturated with 300mL of *S. marcescens* suspended in a solution of 10% sugar water and placed on the bottom of the container. Once the flies were added, they were left to incubate for 24 hours.

Dissections

Crops, once dissected, were soaked separately in 300uL of PBS solution within a petri dish for 48 hours before being washed in a distilled water bath. They were then soaked in a 0.1% Triton X100 solution for 30 minutes before being washed again with PBS and stained with the desired dye.

Staining

Crops were stained with a 0.5% safranin dye that was diluted from a 10% safranin stock in 95% ethanol. Crops were allowed to sit in the safranin for 30 minutes before being removed and washed several times with PBS. Crops were then placed into a droplet of PBS already on a clean microscope slide for examination.

Wild Flies

Wild flies were captured outside of Fernald Hall using generic fly bait and a net trap.

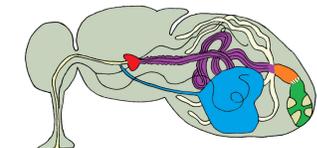


Figure 7: Digestive tract of *Musca domestica* produced by Joyner et al. (2013). The crop is highlighted in blue, the proventriculus in red, the midgut is purple, the hindgut, orange, and the rectum is in green.

Acknowledgements

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- ⁴Joyner C., Mills M.K., Nayduch D. *Pseudomonas aeruginosa* in *Musca domestica* L.: temporospatial examination of bacteria population dynamics and house fly antimicrobial responses. PLoS One. 2013;8