

# Identification by Pupal Weight Using *Cochliomyia macellaria* (F.) and *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae)

Emily Knight and A. L. Brundage  
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## Abstract

In the case of homicides, forensic entomologists are very useful for pinpointing the post mortem interval. In order to accurately do this, they must possess a thorough knowledge of the morphology of blow flies as well as blow fly development. This experiment studies the difference in pupal weight amongst and across the two species of *Cochliomyia macellaria* and *Chrysomya rufifacies* in order to have a comparison. Maggots were collected from carrion and allowed to pupate. Once the pupae emerged, their weights were documented. The blow flies were sexed once they had reached adulthood. It was found that both male and female *Chrysomya rufifacies* weigh more in the pupal stage than do their *Cochliomyia macellaria* counterparts and are larger in size. By using pupal weight to more quickly identify the species, forensic entomologists will ultimately be able to estimate a PMI in a more timely manner.

*Keywords:* blow flies, *Chrysomya rufifacies*, *Cochliomyia macellaria*, pupal weight

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*Chrysomya rufifacies* (Macquart) (Diptera:Calliphoridae) is a relatively new blow fly within the United States, with its first documented identification in 1980. Though this species has not often been reported for playing a role of medical importance, it was recently documented in Thailand as being a myiasis-producing agent (Sukontason 2005). It is known to inflict cutaneous myiasis amongst livestock and is amongst the first species to arrive on fresh carcasses (Byrd 1998). This species is also known to be facultative parasites on other dipteran larvae (Wells and Greenberg 1992a). Being that they share the greatest bionomic similarity with the native species of *Cochliomyia macellaria* (F.) (Diptera:Calliphoridae), *C. macellaria* is a good reference to compare with and several experiments have been conducted that have done so.

An experiment was conducted to study the influence of predator and prey development on predation rates and it was found that though first and second instar *Chrysomya rufifacies* larvae are not predacious, the third instar larvae are predacious and will consume third and second instar *Cochliomyia macellaria* larvae (Wells and Greenberg 1992c). For this reason, the larvae in this experiment will be kept separately so as not to lose any of the test specimens to predation.

A separate experiment was conducted to document the effect that the interaction of these species had on development and rate of development. Though the results conveyed that the sex ratio was not influenced, it was documented that the addition of *Cochliomyia macellaria* increased the time period from eggs to adult in *Chrysomya rufifacies* (Wells and Greenberg 1992b). These results also

contribute to the decision that the larvae in this experiment should be separated by species.

This study analyzed the differences in pupal weight between sexes within each species and the difference in pupal weight amongst the same sex across species. In analyzing these differences, the hope is that forensic entomologists will observe this data and use it to better identify between these species on carrion.

### Materials and Methods

*Chrysomya rufifacies* and *C. macellaria* maggots were collected from carrion found in College Station, Texas and were sorted by species into separate 6.5” by 4.25” clear, plastic Mabis jars (Waukegan, IL) for transport so as to prevent predation. The maggots were then transferred to species specific two gallon rubber Fortex pans (Catano, PR) where they were given food-grade, Grassfed Traditions beef liver (Springville, CA). Once the maggots had grown into flies, these adult flies were collected and set free in a BioQuip 12x12x12 collapsible rearing cage (Rancho Dominguez, CA). The adult flies were supplied with purified water and pure cane, granulated white sugar at all times. When the adults were three days old, food-grade beef liver was again supplied as a protein meal to enable oviposition. When the adults were five days old, new food-grade beef liver was supplied as an oviposition medium.

The eggs laid by the adults were left on the liver while the maggots were allowed to grow. The newly emerged maggots were separated by species, to again prevent predation, and then into groups of three. Each group of three was placed into a one pint, Ball mason jar (Broomfield, CO) atop a bed of white, calcium carbonate sand. Fresh beef liver was added to each of the jars as a food source and was repeatedly added as needed.

Once the maggots pupated, the pupae were collected, sorted into their designated

species and weighed one at a time. After each pupal weight was recorded, the pupae were individually placed into two ounce cups with lids (Diamond) and left at room temperature to let them emerge. The newly emerged adults were then sexed.

Finally, the collected data were analyzed using a T-test in SPSS (LS 2013).

### Results

The findings demonstrate that both male and female *Chrysomya rufifacies* weigh more in the pupal stage than do their *Cochliomyia macellaria* counterparts. They also reveal that the average weight of males is greater than the average weight of the females.

Table 1. This table shows what the calculated average pupal weight of each species was.

Species	Average Pupal Weight
<i>Macellaria</i> males	0.043339
<i>Rufifacies</i> males	0.065225
<i>Macellaria</i> females	0.042606
<i>Rufifacies</i> females	0.061941

Ultimately, a T-test in SPSS was used to analyze the data regarding the comparison

of *Cochliomyia macellaria* to *Chrysomya rufifacies*. T-test P value regarding the male comparison was 1.6E-13 while the value regarding the female comparison was 3.9E-11. Because both of these p-values are much smaller than 0.5, it is indicated that the mean is different from the hypothesized value.

## Discussion

It is known that *C. rufifacies* look much larger than *C. macellaria* just by observation. Therefore, the results obtained were expected, and the difference in size explains the weight difference observed in the experiment.

One might assume that females would weigh more than males being that they are often bigger and must carry the extra weight of ovaries. However, the results demonstrate that this assumption is false.

The average pupal weight difference amongst the sexes is smaller in that of *Cochliomyia macellaria*, than in that of *Chrysomya rufifacies*. When compared, *Chrysomya rufifacies* males are larger than

that of their *Cochliomyia macellaria* counterparts.

*Chrysomya rufifacies* is an important species in terms of forensic importance in that it is among the first insects to arrive at carrion (Byrd 1998). Due to the shortage in biological information about this blow fly (Sukontaskon et. al 2001), this experiment strove to identify the difference in pupal weights amongst the separate sexes within this species and that of *Cochliomyia macellaria*, which is more commonly known. Furthermore, this experiment sought to determine whether distinction could be made between males and females within a species based on their pupal weight and whether distinction could be made amongst these species based on their pupal weight.

These findings can be used to more easily identify between these two species when collecting specimens from carrion. With every new addition of data, forensic entomologists are better able to efficiently do their job knowing that they have a growing information bank at their disposal.

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