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EFFECT OF WATER ON BLOW FLY (DIPTERA: CALLIPHORIDAE) COLONIZATION OF PIGS IN NORTHWEST INDIANA

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ABSTRACT. Forensic entomology is the use of insects in the criminal justice system. Blow flies (Diptera: Calliphoridae) are early colonizers of carrion and any information on factors that influence their oviposition (egg laying) is of vital importance to forensic entomologists. This study examined the effect that being placed in a water environment had on blow fly oviposition. Six pigs were used in this study: three were in water and three were on land (control). Pigs were checked daily to document the arrival time of adult flies, fly eggs, fly larvae, the start of larval migration, and the end of larval migration. Data were analyzed using t-tests to determine if significant differences existed in the timing of blow fly life events between control pigs and pigs in water. Significant differences were seen in the timing of adult flies, fly eggs, fly larva, start of larval migration, and the end of larval migration. Pigs in the water environment initially sank but floated on the water's surface after four days. Colonization by blow flies occurred five days after field placement on the pigs in water. There was an average of a five day difference in postmortem interval (PMI) estimations between control pigs and pigs in water. The results from this study will be valuable to forensic entomologists because it provides important information about blow fly oviposition, growth and development on pigs in a water environment.

Keywords: Blow fly, water, oviposition, forensic entomology

INTRODUCTION

Forensic entomology is the use of insects in the criminal justice system (Greenberg 1991; Haskell & Williams 2008; Byrd & Castner 2010). There are three main areas of forensic entomology: urban, stored product pests, and medico-legal (Catts & Goff 1992; Hall 1995; Byrd & Castner 2010). Medico-legal forensic entomology focuses on the use of insects in determining the amount of time that has passed since insect colonization. Colonization by blow flies (Diptera: Calliphoridae) usually occurs within the first few hours after death and is used to estimate the postmortem interval (PMI) (Haskell & Williams 2008). The PMI is the period of time between death and corpse discovery. Establishing the PMI is important to investigators because it helps limit the number of possible suspects, or validate testimonies.

The PMI is calculated using a system of accumulated degree hours (ADH), or accumulated degree days (ADD). Based on life history characteristics and larval development times of different fly species, ADH (or ADD) is a measure of thermal energy required for insect larvae to reach a specific life stage. The ADH can be applied to determine an approximate time since death (Kamal 1958; Anderson 2000; Byrd & Allen 2001).

A number of different factors can influence blow fly oviposition (egg laying) such as weather, chemicals, and fly access to the corpse. An example of a barrier to oviposition that impacts fly access to a corpse would be an aquatic environment (submersion in water). A body placed in or around water could have an effect on insect colonization and subsequently the PMI. Most aquatic forensic entomology studies focus on aquatic insect species (Haskell et al. 1989; Vance et al. 1995; Keiper et al. 1997) and not blow flies colonizing pigs in water. Tomberlin & Adler (1998) studied the effect of water submersion on rats in the summer and winter months in South Carolina. They found that no flies colonized rats in water during the winter, and three species (*Cochliomyia macellaria* (Fabricius), *Lucilia sericata* (Meigen), and *Sarcophaga bullata* (Parker)) colonized in summer months. The rats in water had a four day delay in oviposition during the summer. Similarly, while conducting research on blow fly colonization on pigs in a manmade pond in Malaysia, Chin et al. (2008) found a four day delay in oviposition on the pigs in water.

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This study examined the effects of a water environment on blow fly oviposition and subsequent life stages. Pigs were used as the carrion model because they have been found to be the best substitute for humans in forensic entomology research studies (Haskell & Williams 2008). Researchers have hypothesized that the pigs in water would have a delay in oviposition that is related to the submersion of the pig.

METHODS

Research was performed in Valparaiso, Indiana, from 23 September — 14 October, 2013. The research area was a clearing in a wooded area on Valparaiso University's campus (approximate GPS coordinates 41°27′39.3″N, 87°03′02.3″W). Research was conducted inside a metal dog kennel measuring 6.1 m × 6.1 m × 1.8 m to prevent predation. Orange snow fencing covered the top of the kennel to allow sun and fly access but deter predators.

Six frozen pigs (*Sus scrofa*) were obtained from Birky Farms in Kouts, Indiana, and thawed for 15 hours in a room without fly access prior to field placement. Bugajski et al. (2011) determined that freezing prior to field placement does not significantly impact blow fly activity. A random number generator was used to place pigs inside of 1 m plots within the research area. Two treatments were examined, pigs that were placed on land (control) and pigs that were placed in water. Each treatment had three replicates. The water environment was created by filling 62 L plastic containers one-half full (31 L) with Valparaiso city water (Fig. 1). Pigs were placed into the container of water at the start of the experiment and stayed in the water for its entirety. Because pigs were not held under the water by weights, they went through periods of submersion and floatation on the surface.

The pigs were checked once daily at 1500 hr to document the arrival time of adult flies, fly eggs, fly larvae, the start of larval migration, and the end of larval migration. Daily samples of adult flies and fly larvae were taken from each pig and preserved in 70% ethanol. Specimens were identified using taxonomic keys (Stojanovich et al. 1962; Whitworth 2008).

Data were analyzed with SPSS® Statistics 18 software using t-tests to determine if significant differences existed in the timing of blow fly life events between control pigs and pigs in water (SPSS 2009). The species compositions of adult and larval flies were graphed.

Figure 1.—Pig in a 62 L plastic container with water. Containers were filled with 31 L of water prior to pig placement.

RESULTS

Significant differences existed in the first appearance of adult flies ($t = -3$, $df = 4$, $P = 0.04$), eggs ($t = -10$, $df = 4$, $p = 0.001$), larva ($t = -11$, $df = 4$, $P < 0.001$), larval migration ($t = -7.18$, $df = 4$, $P = 0.002$), and the end of larval migration ($t = -391.0$, $df = 4$, $P < 0.001$).

The main difference in species diversity between the control and pigs in water was the absence of *Calliphora* and *Ophyra* spp. adult flies on water pigs (Fig. 2). Adult flies on the control pigs were comprised of 47% *Lucilia coeruleiviridis* (Macquart), 20% *Phormia regina* (Meigen), 26% *Ophyra* spp., and 7% *Calliphora* spp. (Fig. 2). Water pigs had less diversity in adult flies and were comprised of 72% *L. coeruleiviridis* and 18% *P. regina* (Fig. 2). Larval diversity was reduced from adult diversity in both treatments. Larval composition on pigs in water was made up of 59% *L. coeruleiviridis* and 41% *P. regina* (Fig. 3). The only larva represented on control pigs was *L. coeruleiviridis* (Fig. 3).

The average temperature during the experiment was 15° C; the highest recorded temperature was 27° C. ADH calculations correctly estimated control pigs to have been in the field since 23 September 2013. However, there was greater variation in the PMI estimations for the water pigs. The estimates for the water pigs were 27—29 September 2013. This represents a

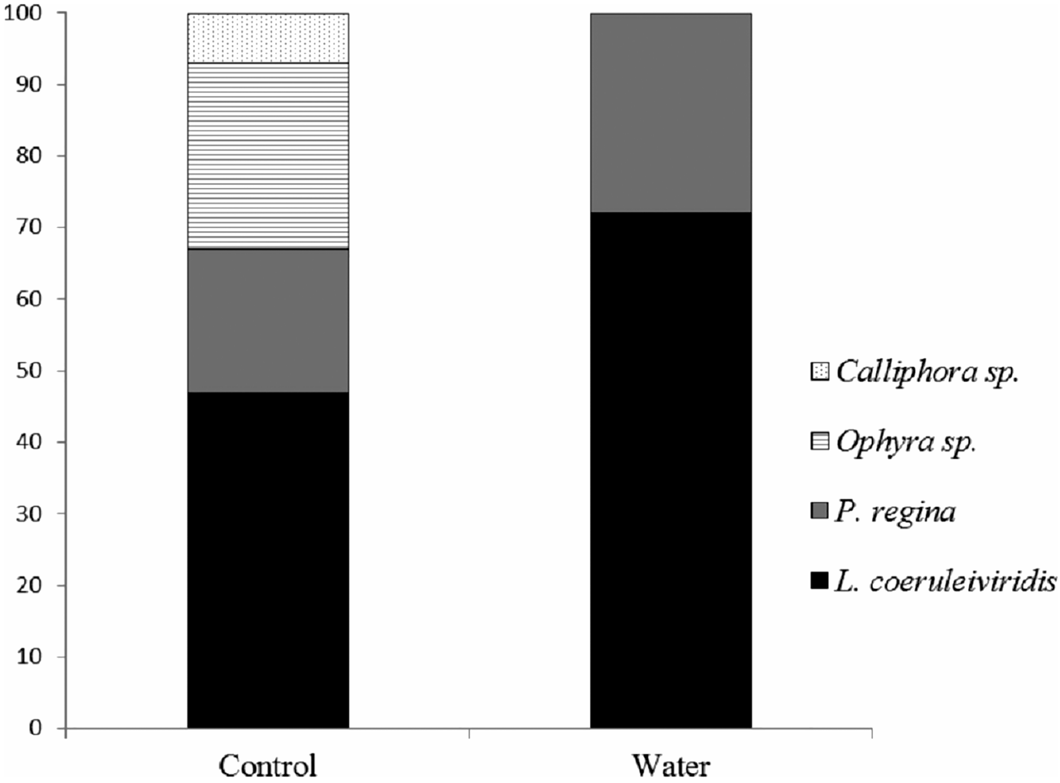

Figure 2.—Species composition of adult blow flies on water and control pigs.

four to six day delay difference from their actual placement.

DISCUSSION

Since decomposition progresses differently than on land, water presents unique challenges for forensic entomologists examining insect evidence. Haefner et al. (2004) characterized the stages of decomposition for submerged pigs as follows: submerged fresh, early floating, early floating decay, advanced floating decay, and sunken remains. They found that it took two to 13 days for the carcass to rise to the surface depending on the water habitat and time of year.

Pigs in this experiment followed the progression outlined by Haefner et al. (2004) and floated after 4 days (Fig. 4). They remained on the water surface for the remainder of the experiment. Colonization by blow flies occurred five days after placement in the field. This is similar to the four day delay in oviposition observed by Tomberlin & Adler (1998) and Chin et al. (2008). Large maggot masses were seen on the floating pigs 12 days after placement (Fig. 5). The initial sinking of the pigs delayed oviposition, but the blow fly life cycle continued the same as control pigs once the water pigs reached the floating stage. Every blow fly life stage was significantly delayed on water pigs when compared to the control. The control pigs were completely skeletonized at the conclusion of the experiment, while the pigs in water had large amounts of soft tissue remaining.

The blow fly life stage most impacted by water was larval migration. On land larvae will migrate into soil cracks or crevices for protection during the pupation stage. In a water environment, larval migration into water results in death either through drowning and decomposition, or through predation by aquatic organisms. The loss of larvae in turn eliminates the long pupal stage that is critical to successful PMI calculations. Kamal (1958) found that the pupal stage accounts for approximately half of the blow fly life cycle, making it the longest of all the life stages. Pupation follows larval migration and water

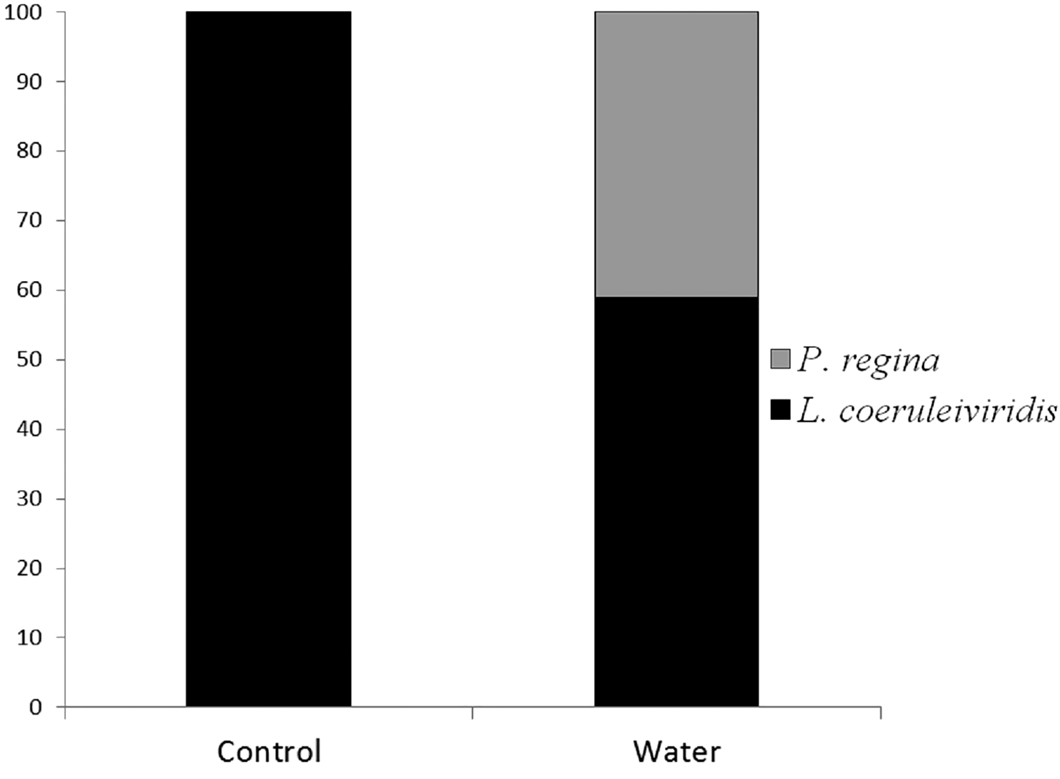

Figure 3.—Species composition of larval blow flies on water and control pigs.

eliminates the possibility of this stage. If a body is found in water and it has been colonized by blow flies that are absent from the body at the time of discovery, forensic entomologists should consider that maggots were present and died during migration.

The difference found in PMI estimations between control and pigs in water is the essential finding in this experiment. The PMI estimations for the pigs in water were an average of five days later than estimations for the control pigs. Furthermore, a greater variability in PMI estimations was seen in pigs in water when compared to the control. The control pig replicates all had the same PMI estimation date, while the pigs in water had

Figure 4.—Pig floating on the water's surface (4 days after placement).

Figure 5.—Large maggot mass on floating pig (12 days after placement).

estimation dates spanning a three day period. Since PMI estimations are based on maggot growth, the timing of oviposition is critical and the significant difference in oviposition timing between water and control pigs is the reason there is a difference.

This research provides important information about blow fly oviposition, growth and development on pigs in a water environment. Due to the variability of decomposition in water, more research needs to be conducted on pigs in a water environment to accurately estimate the PMI. Variables might include water temperature and season of the year, size of the pig, water quality, and depth of the water. Greater understanding of blow fly behavior when water is involved could complement aquatic forensic entomology studies especially when aquatic insects are limited.

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