

Analysis of the Successional Patterns of Insects on the Remains of Methadone-Dependent Rabbits [†]

Davood Keshavarzi

Tehran University, Entomology Department, Iran

* Correspondence: keshavarzd25@gmail.com

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Abstract: The insect successional wave is a primary method to estimate the time elapsed since death. Several studies have shown that antemortem consumption of opioids affects maggot growth rates. However, there are no published data that investigate the effect of antemortem opioids uses on successional patterns. Therefore, the purpose of this research was to investigate the effect of methadone on the successional patterns of insects on rabbit carcasses. During this successional study, 15 and 12 insect taxa were recorded on carcasses for 15 d sampling intervals during spring and winter 2018, respectively. *Chrysomya albiceps* and *Calliphora vicina* were the most frequent fly species. These two species preferred to lay eggs on the control carcasses earlier than the treated carcasses. *Lucilia cuprina* was observed exclusively on the remains of untreated carcasses, while *Saprinus chalcites* was recorded only from the remains of treated rabbits. Permutation analyses based on the Mantel test for the similarity values of taxa between treated and untreated carcasses in the spring and winter were 0.64 ($P = 0.009$) and 0.69 ($P = 0.003$), respectively. Permutation analyses for the two most dominant fly species between the treated and untreated carcasses in the spring and winter were 0.51 ($P = 0.05$) and 0.49 ($P = 0.09$), respectively. The results revealed that the overall pattern of insect succession was similar between the treated and untreated rabbit carcasses. However, the patterns of succession of *Chrysomya albiceps* and *Calliphora vicina* were slightly different between both treated and untreated carcasses and this could have an influence on the PMI estimation.

Keywords: Forensic entomology; insect succession; methadone

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1. Background

Synthetic opioids deaths continue to rise in different parts of the world [1–3]. More than 42,000 deaths due to opioid overdose have been reported in the United States in 2016 [2]. Methadone is a synthetic opioid drug with a high attraction to μ receptors that prescribed for the treatment of opioid addiction [4]. Methadone is a widely used drug in Iran and 1274 related deaths were reported from Tehran during 2009–2015 [1].

In numerous opioids-related deaths, the deceased persons are not discovered until they are in late stages of decomposition. In such cases, forensic entomology is a reliable method to estimate the postmortem interval (PMI) [5]. The degree of development and successional patterns of insects are two main techniques for estimating PMI. By the successional method, the PMI of a corpse is estimated by comparing the composition of corpse insect fauna with the composition of baseline fauna [6]. Necrophagous species include dipteran and coleopteran species are the most important orders for PMI determination in different stages of decomposition [7].

The effects of drugs on the development rate/time of maggots that cause errors in PMI estimation have been reported in different studies, for example; heroin, codeine, and

methamphetamine decreasing larval development time and increasing the larval growth rate [8-10].

The effect of methadone on the development rate/time of maggots has also previously been investigated and found to be responsible for retardation in the developmental time of the *Calliphora vicina* species [11]. However, there are no published data that investigate the effect of antemortem methadone use on successional patterns.

Hence, the current study described here attempts to determine whether antemortem consumption of methadone affects successional patterns of insects on decaying remains.

2. Materials and Methods

2.1. Study Sites

The study was conducted in winter (late-January to March) and spring (late-April to May) of 2018 in the Research Station of Kazerun (29°37'10"N 51°39'15"E), a research facility of Tehran University of Medical Sciences located in Kazerun County in southwest Fars and is

860 m above sea level. The climate in the area is temperate with average temperatures in the spring and winter of 28.0 and 10 ° C, respectively. Average annual rainfall is 257 mm and the most precipitation falls in February, with an average of 62 mm.

2.2. Carcasses and Methadone Dosing

Four rabbits (\approx 1.8 - 2 kg) obtained from the Animal Lab at Shiraz University. Two animals were administered methadone before euthanasia and two were used as controls. To imitate real human methadone consumption, trial rabbits in the first day received 2 mg/ kg/day methadone orally by gavage and then the dosages were gradually increased to 20 mg/ kg/day over two weeks. At the end of the period, to ensure rabbits are dependent to methadone, naloxone (0.5 mg/kg i.m.) was injected and opiate withdrawal signs (i.e. ptosis and teeth chattering) were observed. At the end of the treatment period, the animals were killed by chloroform in the morning and placed in separate wire cages (75×50×65 cm) with a 2 cm steel-welded tubing, which was surrounded with 1.3 cm mesh. The bottoms of the cages were directly in contact with the ground. The cages were placed in an outdoor site of a research station approximately 80m apart and allowed to decay. All cages received direct sunlight most of the day. Permission to use rabbits as the research animal for the present study was granted by the Ethical Committee of Tehran University of Medical Science.

2.3. Sampling Protocol and Statistical Analysis

Sampling of insects was conducted daily (between 13:00 -16:00 hr) during winter and spring seasons. In the spring, sampling was done twice daily, for fresh and blot stages of decomposition and then once per day for the next stages, in the winter, sampling was done once per day, for 4 days and then twice daily, for the next days. This was done in accordance with Tabor et al study [5]. Samples of adult beetles and maggots were collected, using pitfall traps and forceps. In the present study, immature stages of flies were chosen for the study of succession patterns and during each visit, adult flies activity was recorded. Approximately 20 larvae were collected from each maggot mass during samplings and then they were put in hot water to die. Another group of 20 larvae was collected for rearing to the mature stage. Different taxonomic keys were used for the identification of insect samples [12-15].

Jaccard similarity coefficients were used to determine the match of species between sampling intervals within the experimental carcasses. The Jaccard index ranges from 0-1 demonstrating complete dissimilarity to perfectly match between sampling intervals for any insect species [5]. Average similarities of species between the sampling intervals were calculated for both treated and control groups. Then, similarities were tested using a permutation analysis. In the present study, the H-null hypothesis representing dissimilarity between the successional waves of species from testing and control carcasses ($H_0: P=0$).

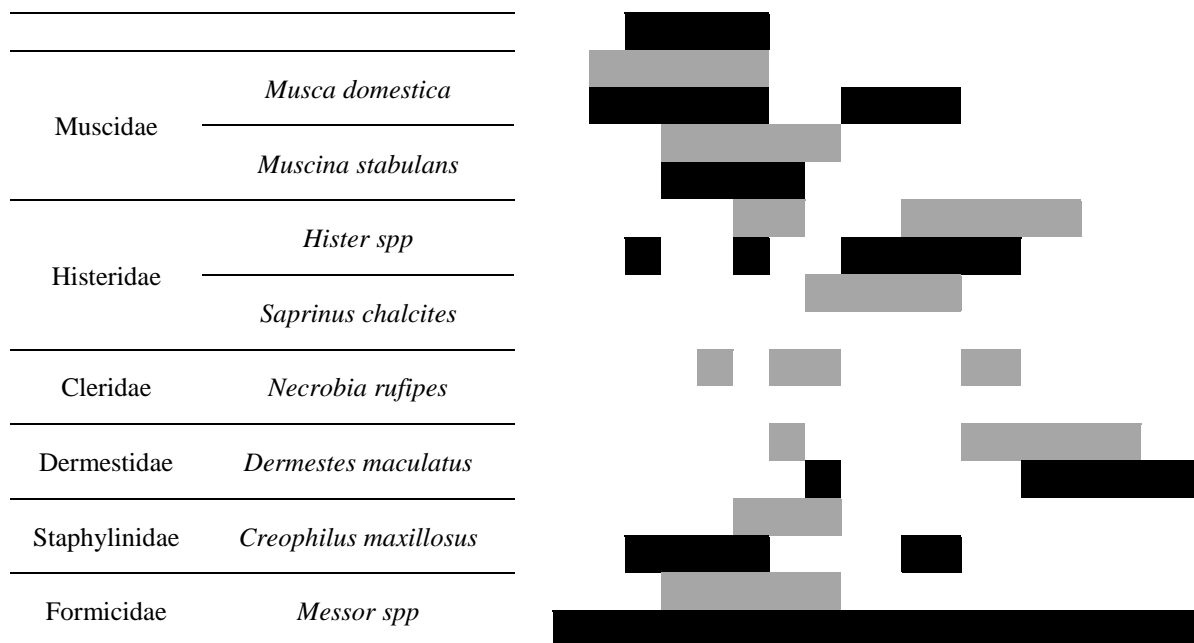


Table 2. Insect succession pattern for Methadone-treated and Untreated rabbit carcasses during 15 sampling intervals in winter 2018.

Family	Species	Sampling Interval (Days)														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Calliphoridae	<i>Calliphora vicina</i>															
	<i>Chrysomya albiceps</i>															
	<i>Lucilia sericata</i>															
	<i>Lucilia cuprina</i>															
Sarcophagidae	<i>Sarcophaga aegyptica</i>															
	<i>Sarcophaga africa</i>															
	<i>Sarcophaga spp</i>															
Muscidae	<i>Musca domestica</i>															
Histeridae	<i>Saprinus spp</i>															
Staphylinidae	<i>Creophilus maxillosus</i>															
Centipedes	<i>unknown</i>															
Formicidae	<i>Dolichoderinae</i>															

Twelve taxa of dipterans in 3 families were demonstrated in the study. Ten of the dipteran taxa were found on both treated / untreated carcasses; however, two of them (*Sarcophaga spp* , *Lucilia cuprina*) were observed only on untreated carcasses. *Chrysomya*

albiceps and *Calliphora vicina* were the dominant fly species in the spring and winter, respectively, and comprising >35% of the specimens collected. Dipteran species were the first visitors to carcasses during spring and winter. *Chrysomia megacephala* was restricted to spring and observed on the remains of both treated and untreated rabbits. The most frequent dipteran larvae were found on untreated carcasses, but the most activity of adult flies was observed in the vicinity of the treated carcasses during fresh and bloat stages. No significant difference was found when comparing the mean number of insect species on treated and untreated carcasses by Mann–Whitney U test ($U = 86$, $z = 1.071$, $P = 0.28$). Diversity analysis indicates high species richness and species diversity in the spring (Shannon = 2.5; Margalef = 2.2). But, the diversity t test analysis on the Shannon index showed no significant difference between spring and winter 2018 ($P < 0.05$).

Coleopterans were demonstrated by 6 taxa in 4 families. Four of the beetle species were collected from both treated and untreated carcasses. *Creophilus maxillosus* was the most dominant beetle species comprising >60% of the beetle specimens collected. *Saprinus chalcites* and *Necrobia rufipes* were recorded only from the remains of treated rabbits.

Creophilus maxillosus was recorded only from the remains of untreated carcasses during winter.

Formicidae found mostly on the control carcasses with large numbers at early stages of decomposition. Formicidae predated on the eggs and take pieces of carcass parts as food. *Messor spp* observed on untreated carcasses until the end of the experiment in spring, with their numbers steadily decreasing.

Mean pairwise taxa similarities (Jaccard coefficient) for the successional waves of insect taxa in each sampling period are presented in Figures 2 and 3. The taxa resemblance values for 15 d sampling intervals during the spring trials ranged from 0.0 – 0.28 and 0.05 – 0.33 for untreated and treated carcasses, respectively. Those resemblance values during the winter trials ranged from 0.0 – 0.33 and 0.0 – 0.31 for untreated and treated carcasses, respectively. The lowest resemblances in taxa were observed in the first day of decomposition, but the greatest resemblances were recorded in days 3-5. Correlation analysis was used to test species resemblance values among sampling intervals. Permutation analysis based on the Mantel test between the similarity values of taxa for both treated and untreated groups in the spring and winter were 0.64 ($P = 0.009$) and 0.69 ($P = 0.003$), respectively. So, correlations were significant at the 0.05 level, therefore, the null hypothesis was rejected. Permutation analysis of the similarities was 0.82 ($P < 0.001$) between spring and winter. This result suggest that the successional patterns of insects were similar between spring and winter.

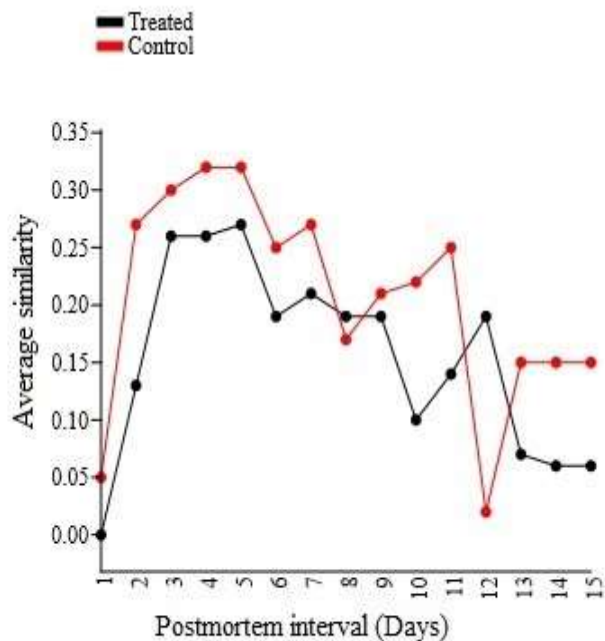


Figure 2. Mean pairwise Jaccard similarities for each sampling interval during the succession of insect species on methadone-treated and untreated carcasses in spring 2018.

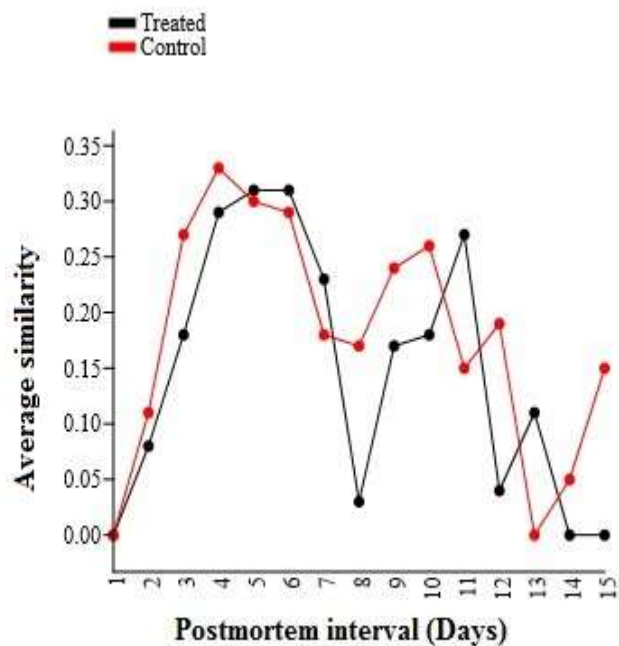


Figure 3. Mean pairwise Jaccard similarities for each sampling interval during the succession of insect species on methadone-treated and untreated carcasses in winter 2018.

Permutation analyses between the similarity values of the two most dominant fly species (*Chrysomya albiceps* and *Calliphora vicina*) for both treated and untreated carcasses in spring and winter were 0.51 ($P = 0.05$) and 0.49 ($P = 0.09$), respectively. Interestingly, this result showed that the successional patterns of those two species on the remains of both treated and untreated rabbits were dissimilar, but colonization of them was similar. Those two species prefers to lay eggs on the control carcasses earlier than the treated carcasses.

4. Discussion

In the present study, decomposition of the control carcasses was faster than the treated carcasses, because of the more number of insects present. This finding is similar to Rodriguez and Bass and Abd El-bar and Sawaby studies [18, 19]. Not amazingly, calliphorid flies were the first visitors to both carcass. This observation was in agreement with previous studies [16, 20]. *C. vicina* and *Ch. albiceps* were the dominant species and constituted the primary colonizer in spring and winter, respectively. Abd El-bar and Sawaby reported that *Ch. albiceps* is a dominant species and also a primary visitor on carcasses of rabbits treated with an organophosphate insecticide [19]. In this study, we used maggots to study the succession pattern. According to Smith study, the occurrence of adult fly species on the remains doesn't necessarily mean that egg laying or larviposition is occurring [21]. The most frequent of dipteran larvae were found on untreated carcasses, but the most activity of adult flies was observed in the vicinity of the treated carcasses during the fresh and bloat stages. No significant difference was found after comparing the mean number of larvae between both untreated and treated groups ($P = 0.28$), just contrary to Abd El-bar and Sawaby study for organophosphate treated carcasses [19]. Adult female flies select safe sites for larviposition because of the presence of the xenobiotics, predators, and competitors [22, 23]. Mahat indicated that Malathion, in general, delays initial oviposition of dipteran species [23]. Therefore the most activity of adult flies in the vicinity of the treated carcasses may be due to their efforts to choose the right breeding site for larviposition. The taxa resemblance values for 15 d sampling intervals ranged from 0.0 – 0.31 and 0.05 – 0.33 for untreated and treated carcasses, respectively. These results showed the low similarity in species among sampling intervals within each group, just as Tabor et al. study. for ethanol-treated and untreated pigs [5]. The low similarity degree revealing rapid temporal changes in taxa composition. Therefore, the slowest turnover in taxa composition occurred on days 4-7. The overall pattern of insect succession was similar between the treated and untreated rabbit carcasses ($P < 0.05$). In parallel, Tabor et al. found that successional patterns of insect species were similar between ethanol-treated and untreated carcasses [5]. Similar findings has been reported in previous studies for organophosphate treated carcasses [19, 24]. In the present study, statistical analysis showed that the succession patterns of the two most dominant fly species (*Ch. albiceps* and *C. vicina*) were different between treated and untreated carcasses. This suggested that the ante-mortem consumption of methadone influenced on the occurrence of those species on the carcasses. According to Tabor et al. study, the *C. vicina* species preferred to colonize the ethanol - treated carcasses than the control carcasses [5]. Kolver reported that burning had a significant influence on the succession of *Ch. albiceps* on the carcasses [25]. Differences in the successions of those two species between the carcasses could be due to the effect of methadone on the time of oviposition.

Both *Ch. albiceps* and *C. vicina* species, mostly occur during cooler seasons and they are also a first wave invader during the decomposition of carcasses [16, 26].

5. Conclusions

We conclude that the overall pattern of insect succession was similar between the treated and untreated rabbit carcasses. But the patterns of the succession of two most dominant fly species (*Ch. albiceps* and *C. vicina*) were different between both treated and untreated carcasses. Those two species prefers to lay eggs on the control carcasses earlier than the treated carcasses, and this could have an influence on the PMI estimation based on succession pattern. The current study supplied a database in the field of medico-legal entomology, these data could be useful or estimating minimal postmortem intervals.

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