

Title



**Effects of Antemortem Consumption of Methadone
on Insect Successional Patterns; Implications for
Estimating Postmortem Interval**

Presenter

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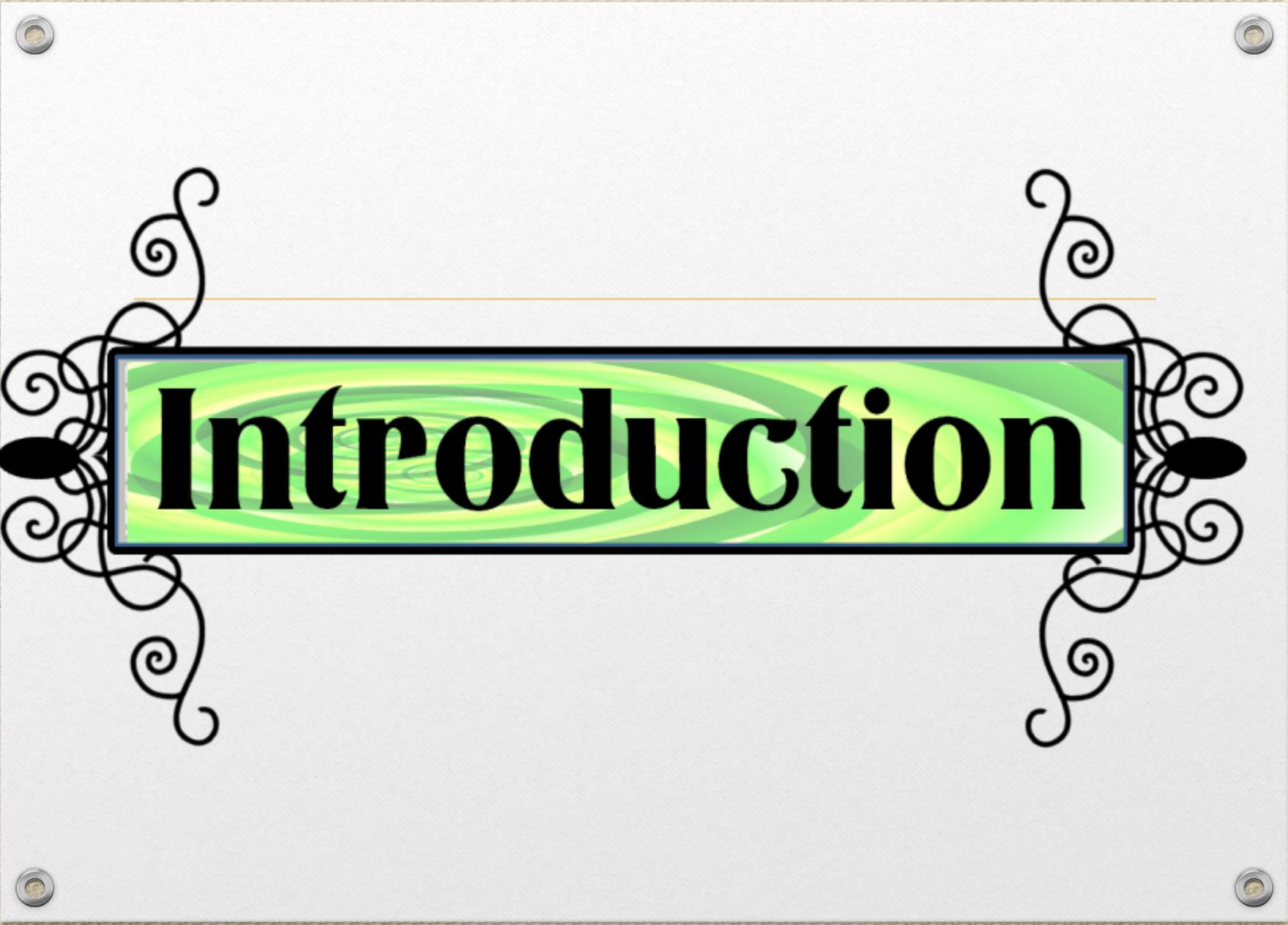


Prof. Koroush Azizi



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.



Introduction

Forensic entomology

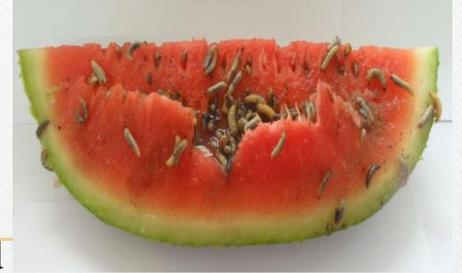
Is insect and other arthropod biology applicable and investigated in criminal matters



3 Broad Categories:

❑ Forensic entomology in urban contexts

For example, a **butcher's shop in London**, was closed in January 2010 and **£560** punished (meat infested with **maggots and fly eggs**) (Gennard,2012)



❑ Stored product contexts

For example, canned citrus juice, a maximum of five or more eggs of *Drosophila* or other insects per 250 ml is allowable (Gennard,2012)



❑ Medico-legal context



History

- Dates back to at least the **13th century** - **Sung Tzu**

Book: Collected Cases of Injustice Rectified



- **Francesco Redi**

In 1668, he disproved the theory of spontaneous generation



- **Bergeret**

- ✓ He was the first to apply forensic entomology to a case

- **Mégnin**

- ✓ successions of insects on corpses



Applications

1- Determination of the postmortem interval



2- Relocation of a corpse

- ✓ For example, **Philonthus Lepidus (Staphylinidae)** was collected exclusively on carcasses in forests in Poland



3- Abuse/Neglect of children/elderly

- ✓ Some species of flies (such as *L.sericata*) are drawn to odors, such as ammonia, resulting from urine or fecal contamination.



4- Proving the identity of the victim

- ❖ In certain cases, when maggots but no corpses are found



- ✓ Isolation of DNA from the **crop** (digestion does not occur) of larva



5- Determine the cause of death

- ✓ Shock caused by a bee sting
- ✓ Drug abuse
- ✓ Detection of toxins



Five Stages of Body Decomposition

1. Fresh
2. Bloat
3. Active Decay
4. Advanced Decay
5. Dry (skeletal)



Examples of Diptera in Early and Late Stages

Early Stage Decomposition



Calliphoridae



Sarcophagidae

Late Stage Decomposition



Piophilidae

Examples of Coleoptera (Beetles)

Early Stage Decomposition



Carrion Beetles (*Silphidae*)

Early to Late Stage Decomposition

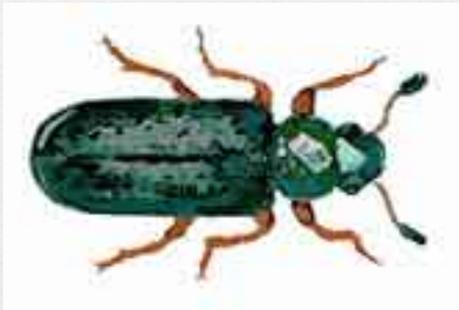


Staphylinidae



Histeridae

Late Stage Decomposition



Cleridae



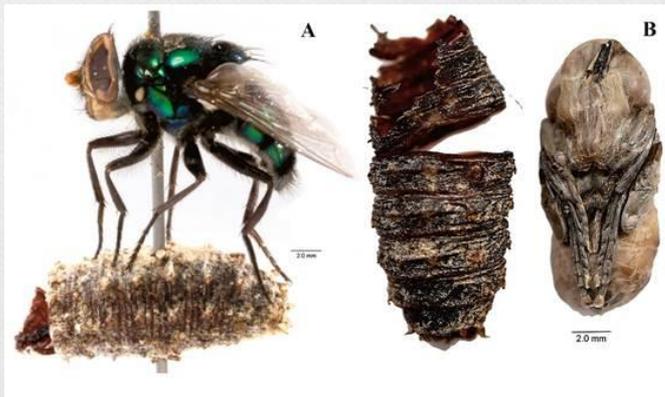
Dermestidae



Scarabidae

Entomotoxicology

□ **Entomotoxicology** is the analysis of drugs and toxins in arthropods (mainly flies and beetles) that feed on carrion



Entomotoxicology

➤ The history of entomotoxicology is relatively short

- ✓ **Beyer and his partners** demonstrating the presence of phenobarbital in larvae found on a skeletonized female body in 1980
- ✓ In 1982, Nuorteva described the recovery of mercury from calliphorid maggots fed on fish tissues containing known concentrations of mercury

Importance

1-Drug abuse detected

- ✓ **Triazolam**, was detected in maggots and not in organ tissue samples of a corpse in France (Introna,2001)
- ✓ **amitriptyline and nortriptyline** were detected from **larvae and exuviae** in Diptera and beetles (Miller,1994)

2-Aid determination of corpse origin

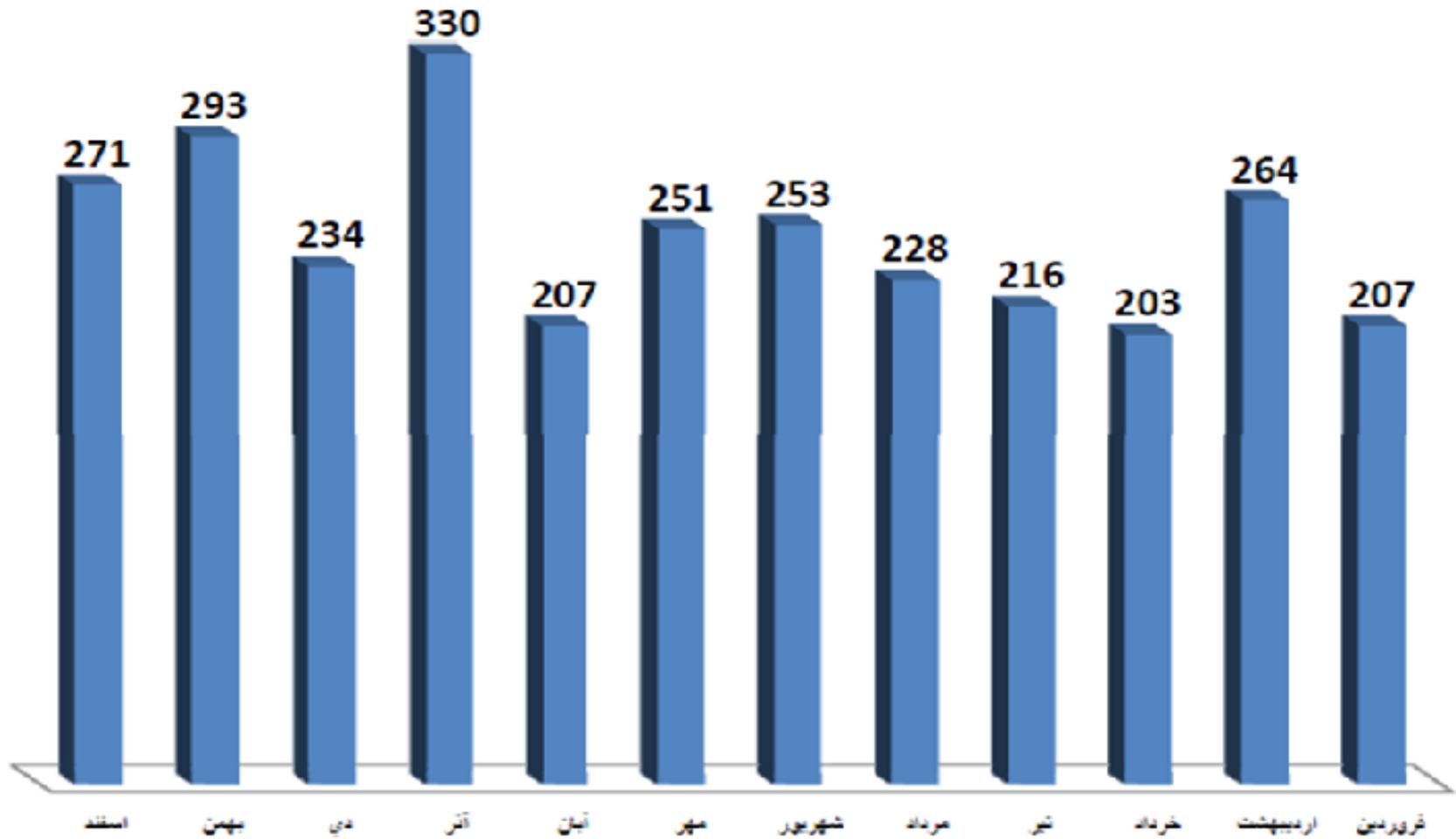
- ✓ **Low levels** of **mercury** in **Larvae collected from** a woman corpse in Finland indicating that the woman **came from an area of comparatively low mercury pollution** (Goff,1994)

3- Confounding of postmortem interval estimate

- ✓ **Effects on development rates of arthropods.**

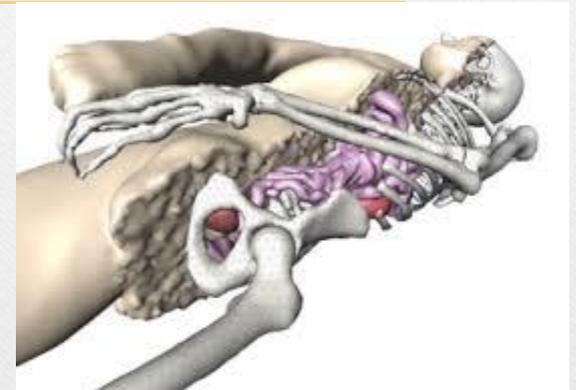


Narcotic drug-related death in Iran-2013



Estimation of the time of death

- **Lividity**
- **Rigor and algor mortis**
- **Changes in the chemical constituents of body**
- **Autolysis of tissue**
- **Entomological evidence**



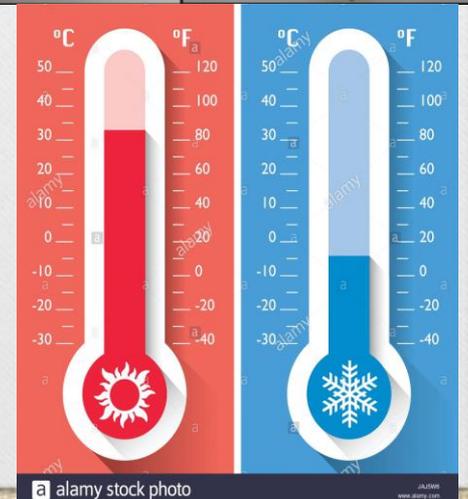
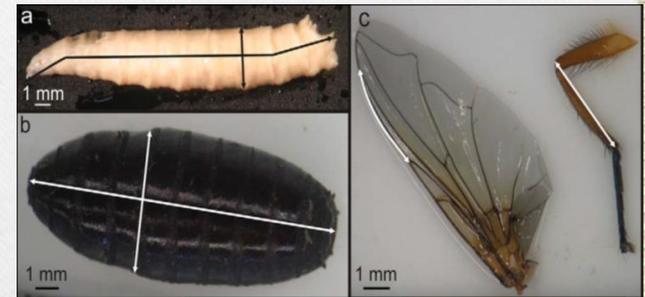
Entomological evidence

1- The degree of development of the oldest maggots

2- Insect successional patterns

These methods affected by;

- ✓ Temperatures
- ✓ Drugs
- ✓ Wrapping
- ✓ Size
- ✓ Location





Materials **AND**
Methods

Study Site

Kazerun Health Research Station

During the winter and spring



Animal model



Male rabbits ($\approx 1.8 - 2.5$ kg)

Eight rabbits in each season, four animals were administered drugs prior to euthanasia and four were used as controls



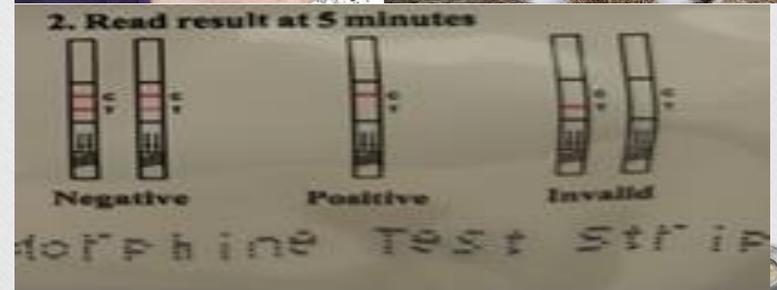
Drug dosing for succession

In order to imitate real human **Methadone** use, on the first day, rabbits obtained **2 mg/ kg/day** methadone orally by gavage and then gradually increased the dose to **20 mg/ kg/day** over two weeks

Addiction

To ensure rabbits are dependent to drugs, **naloxone** (0.5 mg/kg I.M.) was injected

Rapid test for drug detection



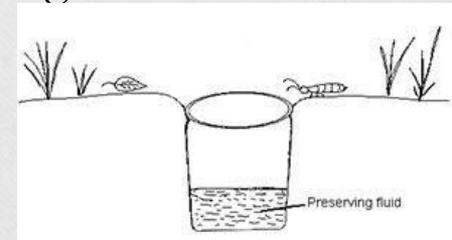


Protocol for Sampling

Sampling of insects was conducted twice daily (between 13:00 -16:00 hr) during seasons ([Tabor *et al.*, 2005](#))

Samples of beetles and flies were collected, using **pitfall traps**, **hand net** and **forceps**

Approximately a group of 30-50 larvae were collected from each maggot mass during samplings and then to death put in hot water, and another group of 20 larvae were collected for rearing to the mature stage





Identification of insect samples

Different approved taxonomic keys were used (*Velásquez et al., 2010*; *Akbarzadeh et al., 2015*; *Ghahari et al., 2015*; *Grzywacz et al., 2017* and ...).

Andreas Herrmann for beetles

Deutschland (Germany)



Effect of drugs on the development rate

Eight rabbits were administered methadone and opium over a period of 14 days, and two rabbits which did not receive drug, were used as a control

Methadone	0 mg/kg (Control)	0.1 mg/kg	0.5 mg/kg	1mg/kg	10 mg/kg
Opium residue	0 ml/kg (Control)	0.5 ml/kg	1 ml/kg	2.5 ml/kg	5 ml/kg

Statistical Analysis

We used the sample-size based rarefaction and extrapolation (R/E) curves with 95% confidence intervals based on a bootstrap method for comparison of species diversity between seasons. The analysis was performed with an R package (iNEXT)

Data normality was inspected using Kolmogorov–Smirnov normality tests. One-way ANOVA test or K.Wallis were used to investigate differences between treatment groups.

RESULTS

A 3D rendering of the word "RESULTS" in large, colorful, block letters. The letters are: R (cyan), E (red), S (magenta), U (orange), L (blue), T (yellow-green), and S (cyan). Small, white, stylized human figures are positioned around the letters, appearing to push or hold them in place. The scene is set on a white reflective surface against a white background. The entire image is framed by a white border with four circular fasteners at the corners.



Results

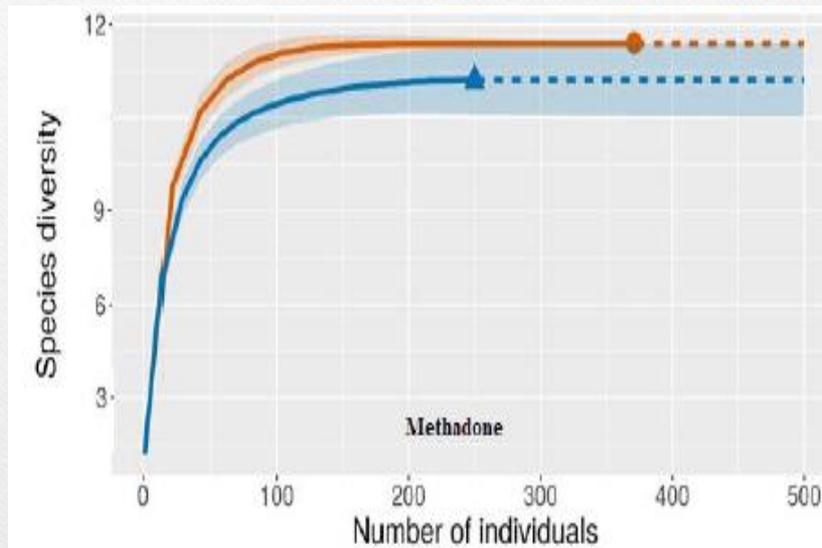


During this successional study, 15 and 12 insect taxa were recorded on carcasses for 15 d sampling intervals during spring and winter 2018, respectively. *Chrysomya albiceps* and *Calliphora vicina* were the most frequent fly species.

Insect species found on Methadone treated and untreated rabbit carcasses- Spring , 2018

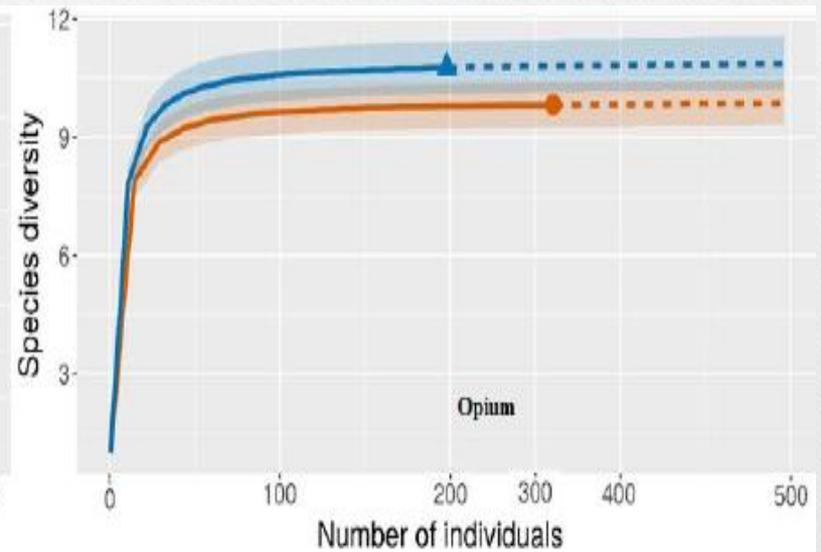
		Sampling Interval (Days)														
Treated	Control	1	2	3	4	5	6	7	8	9	10	11	12	13	14	>14
Calliphoridae	<i>Calliphora vicina</i>	[Grey bar from day 1 to 11]														
	<i>Chrysomya albiceps</i>	[Black bar from day 3 to 12]														
	<i>Chrysomya megacephala</i>	[Grey bar from day 3 to 11]														
	<i>Lucilia sericata</i>	[Black bar from day 1 to 10]														
Sarcophagidae	<i>Sarcophaga aegyptica</i>	[Grey bar from day 5 to 10]														
	<i>Sarcophaga argyrostoma</i>	[Black bar from day 6 to 12]														
	<i>Sarcophaga sp</i>	[Grey bar from day 7 to 11]														
Muscidae	<i>Musca domestica</i>	[Grey bar from day 1 to 11]														
Histeridae	<i>Hister sp</i>	[Black bar from day 3 to 12]														
	<i>Saprinus chalcites</i>	[Grey bar from day 9 to 14]														
Cleridae	<i>Necrobia rufipes</i>	[Grey bar from day 5 to 12]														
Dermestidae	<i>Dermestes maculatus</i>	[Black bar from day 7 to 14]														
Staphylinidae	<i>Creophilus maxillosus</i>	[Grey bar from day 1 to 10]														
Formicidae	<i>Messor meridionalis</i>	[Black bar from day 1 to 14]														

Winter , 2018



— interpolated - - - extrapolated

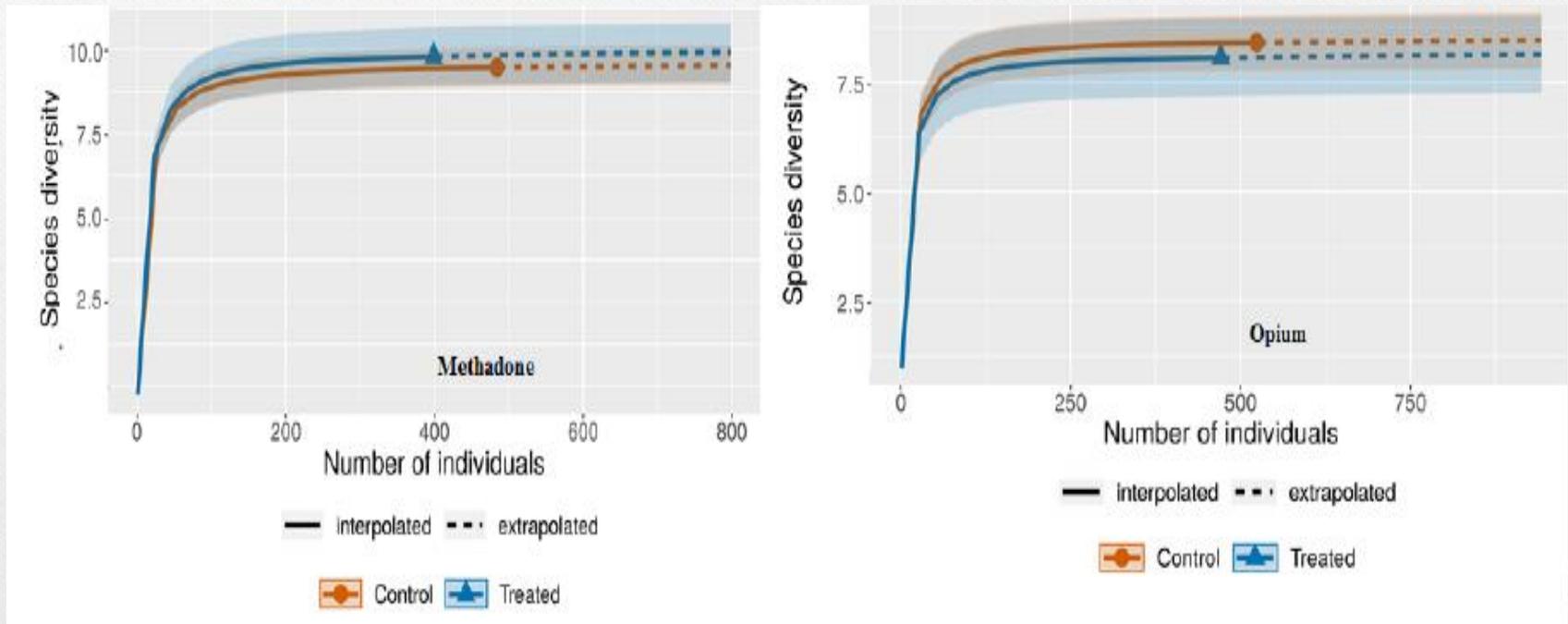
Control Treated



— interpolated - - - extrapolated

Control Treated

Spring , 2018

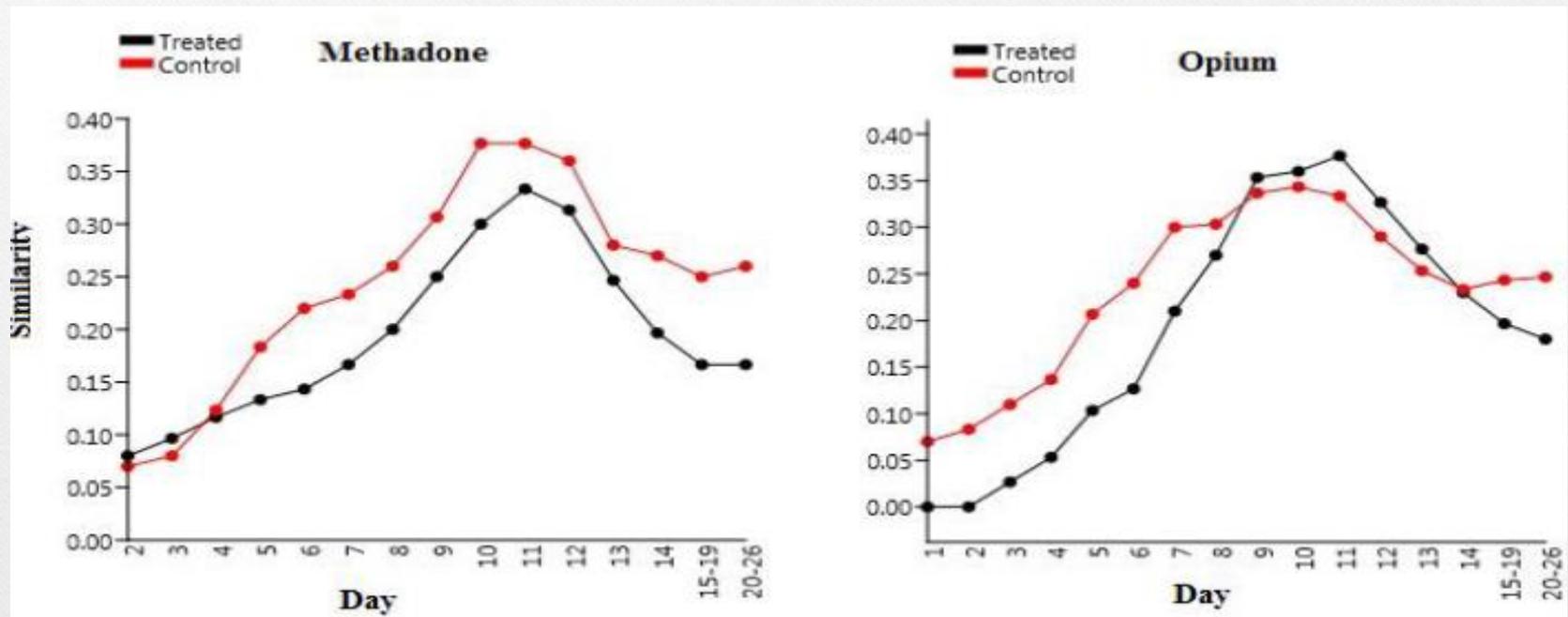


Different stages of rabbit decomposition , 2019



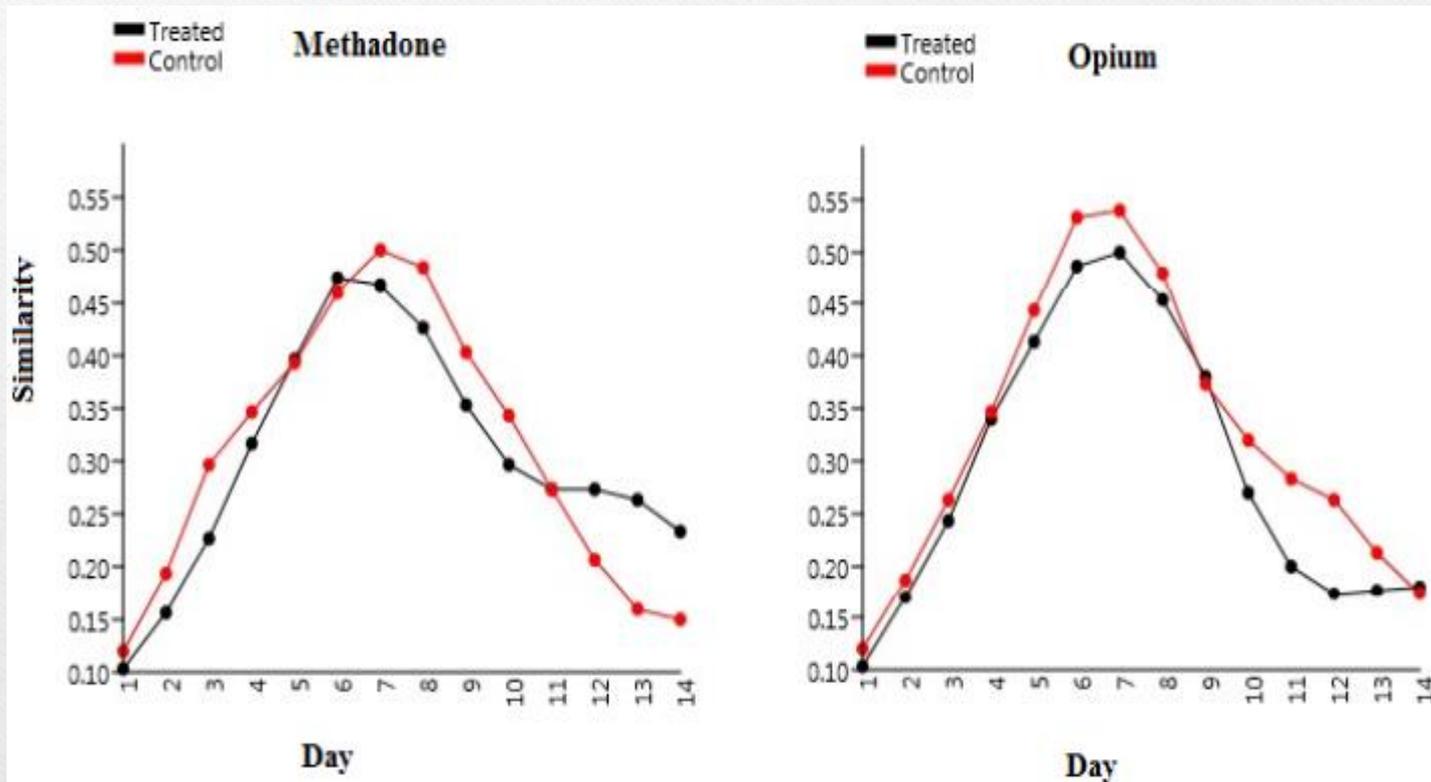
Jaccard similarity

Winter, 2018



Jacard similarity

Spring , 2018



PMI error due to presence
of drugs in carrion

Methadone, up to 24 h

Opium, up to 12 h

Discussion

Dominant species

Adult of calliphorids were the first visitors to both carcass, this observation was in agreement with [Mashaly & Al-Mekhlafi, 2016](#). *Calliphora vicina* and *Ch. albiceps* were the dominant species and constituted the primary colonizer in warmer and cooler seasons, respectively. This observation was in agreement with [El-Bar & Sawaby, 2011](#), [Salimi et al , 2018](#)

Succession

Permutation analyzes based on the Mantel test and the Jaccard similarity index found that the overall sequence of insect succession during **winter** varied between the **methadone** treated and untreated rabbit carcasses.

Succession

- Abd El-bar and Sawaby (2011) found that the **mean number** of collected larvae per rabbit-visit from control carcasses significantly exceeded those collected from the remains treated with an **organophosphate** insecticide
- A field evaluation of the effect of **malathion** on the pattern of insect succession found that this insecticide altered the rate of **decomposition and the diversity of species** (Yan 2010)

- ❑ Tabor et al.(2015). found that successional patterns of insect species were similar between **ethanol-treated** and untreated carcasses
- ❑ Abouzied (2016), observed that **tramadol** treated carcasses are more attractive for females of some fly species
- ❑ Therefore, it seems that the presence of flies on carcass could be affected by the drugs in the carcass tissues.

- ❖ Gosselin (2011) detected methadone from *L. sericata* puparial cases at treatment 0.8 mg/g and 4 mg/g but not at 0, 0.2 mg/g and 0.4 mg/g

- ❖ Methadone puparial case concentrations are 60 times lower than methadone concentration in 3rd instar larvae
- ✓ In this respect, the cuticle acts as storage organ, similar to adipocytes and pericardial nephrocytes (Bourel, 2001)
- ❖ No correlation between methadone concentration in a single puparial case and in its substrate is observed

Most important collected species

Histeridae

Saprinus chalcites

Hister sp



Dermestidae

D. frischii



D. maculatus



C. vicina



Ch. albiceps



L. sericata



CONCLUSION

Conclusion

Finally, we conclude that the overall pattern of **insect succession was similar** between the treated and untreated rabbit carcasses during spring

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. .

Acknowledgment

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thank
you