The Effects of the Freeze and Thaw Cycle on the Process of Decomposition and Entomological Succession: Preliminary Results

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Introduction

Decomposition refers to the process that is initiated upon death and continues until the skeleton is exposed. The stages most commonly cited are fresh, bloat, active decay, advanced decay, and dry remains or skeletonization[1]. The rate of decomposition is influenced by many factors, including temperature and the presence of insects[2]. As temperature increases, the activity of microbes, invertebrates and vertebrate scavengers increases too. In contrast, colder temperatures slow down and may even halt these activities. The field of forensic entomology has mainly focused on insect succession during warmer temperatures, with only a few studies performed in cold temperatures and cold climates. Therefore, the objective of this study was to investigate the freeze and thaw cycle on the decomposition process and insect succession in

Experimental

- A pilot study was carried out using three pig carcasses placed on the Université du Québec à Trois-Rivières campus on February 25, 2020 (day 0). Data loggers were placed in the mouth and rectum to measure the internal body temperature, and another data logger placed on the anti-scavenging cage to record the ambient temperature. Initially, sampling occurred once a week. Once temperature and insect activity increased, sampling occurred every day, then every second day, twice a week, and finally once a week until October 23 (day 242).
- Ground-dwelling insects, larvae, and eggs of Diptera were collected using forceps and gloved fingers. Three pitfall traps were placed a meter away from the head, abdomen, and rear of each carcass. Flying insects were collected by swinging an entomological net over the carcasses. All adult insects were killed and preserved by freezing. Fly eggs and larvae were collected, reared to adulthood, and identified to the species level.
- The rearing was performed in Mason jars filled with wood chips, and an aluminum cup containing pork liver. The eggs and larvae were placed directly on the liver and moistened every day[3]. The jars were placed in an environmental chamber set to 23°C with humidity of 70%, a photoperiod of 8:16 (light:dark) for the winter time, then 12:12 once the summer started. The emerged adults were collected and frozen. All the insects were identified using identification keys and a stereomicroscope.

Table 1: Diptera and Coleoptera observed on carcasses in 2020

Diptera	Coleoptera
First Diptera seen: Muscidae, Day 53 (18 April, 7°C)	First Silphidae seen: Day 64 (29 April, 17°C)
First Diptera eggs seen: Day 53 (18 April, 7°C)	First Styphilidae seen: Day 68 (03 May, 17°C)
First Calliphoridae captured: Day 59 (24 April, 9°C)	First Nitidulidae seen: Day 76 (11 May, 9°C)
First Diptera larvae seen: Day 67 (02 May, 12°C)	First Cleridae seen: Day 83 (18 May, 17°C)
First Sepsidae seen: Day 68 (03 May, 17°C)	First Histeridae seen: Day 102 (06 June, 19°C)
First Piophilidae seen: Day 78 (13 May, 12°C)	
First Sarcophagidae seen: Day 85 (20 May, 18°C)	
First Diptera pupa seen: Day 96 (31 May, 9°C)	

Table 2: A 'snapshot' of insects captured and identified between April 24 to May 3, 2020

Date of Capture	Pig #	Genus/Species
24 April 2020 (Day 59)	A	*Calliphora* sp.
	C	*Calliphora* sp.
25 April 2020 (Day 60)	A	*Calliphora* sp.
		Phormia regina
29 April 2020 (Day 64)	B	*Calliphora* sp.
	C	*Protophormia* sp.
1 May 2020(Day 66)	C	*Calliphora* sp.
2 May 2020 (Day 67)	A	*Protophormia* sp.
3 May 2020 (Day 68)	A	*Phormia regina*

Figure 2: ***Phormia regina***

Figure 3: ***Calliphora sp.***

Results

Decomposition process

All pig carcasses were in the fresh stage at the beginning of the study (day 0). Pig B entered the bloat stage on day 71, while Pig A and C bloated on day 86. Active decay was observed on Pig B on day 91, and on Pigs A and C on day 93. Pigs A and C entered advanced decay on day 97, while the front half of Pig B entered advanced decay on day 94, and the whole body on day 105. Signs of dry remains and/or skeletonization were observed on day 102 for Pig B, and on day 114 for Pig A and C. By day 155, all carcasses were in the final stage of decomposition until day 242. **Figure 1** highlights the visual stages of decomposition.

Temperature

The minimum recorded ambient temperature was -1°C (March 23) and the maximum recorded temperature was 43°C (June 2).

Arthropods

Data presented here were gathered from the entomological net and visual observations on the carcasses (Tables 1 and 2). First Diptera of forensic interest (Muscidae) was observed on the carcasses on April 18 (Day 53), when the ambient temperature was 7°C, while the first Coleoptera of forensic interest (Silphidae) was observed on April 29 (Day 64) when the ambient temperature was much higher (17°C). To date, 9 Diptera and 5 Coleoptera families have been identified, but identification is still ongoing, and the rearing data has yet to be analyzed.

Discussion

The results of this study were similar to a summer study conducted in 2019 at the same site[4]. Several differences were however observed between the summer and winter studies, particularly relating to the decomposition rate and process. For example, during the summer study, the bloat stage commenced on day 2 and lasted for five days, while in the winter study, this stage commenced on day 86 and lasted 20 days. In addition, the active decay stage was observed from day 7-13 in the summer study, while this stage occurred much later (from day 93-97) in the winter study but transitioned more quickly. Therefore, it appeared that the decomposition process starts much later in colder temperatures, but once initiated, as temperatures increase, the stages transcend more quickly than in summer.

The insect succession of both studies were globally similar with however, some differences. The main difference was a delayed colonization of the Diptera and Coleoptera in the winter experiment which arrived on day 53 and 64 respectively, compared to the summer experiment which arrived on day 0.

Identification of all the species caught (especially data from the rearing) will provide more information about the succession of Diptera and Coleoptera. The preliminary results of this study show that the freeze and thaw cycle causes changes to the decomposition process and entomological succession, but further analysis of the reared Diptera, captured Coleoptera and Diptera, and meteorological data is needed to make an accurate conclusion.

Figure 3: Stages of decomposition for the winter 2020 study.
Left to right: A) Fresh (Day 0), B) Bloat (Day 91), C) Active decay (Day 93), D) Advanced decay (Day 101), E) Dry Remains (Day 175)

References

[1]Payne J. A. Summer Carrion Study of the Baby Pig *Sus Scrofa* Linnaeus. Ecology 1965; 46(5):592-602.
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