JOURNAL OF FORENSIC SCIENCES

J Forensic Sci, January 2011, Vol. 56, No. 1
doi: 10.1111/j.1556-4029.2010.01552.x
Available online at: interscience.wiley.com

PAPER

PATHOLOGY/BIOLOGY

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Effect of Investigator Disturbance in Experimental Forensic Entomology: Carcass Biomass Loss and Temperature

ABSTRACT: Often carrion decomposition studies are conducted using a single carcass or a few carcasses sampled repeatedly through time to reveal trends in succession community composition. Measurements of biomass and other abiotic parameters (e.g., temperature) are often collected on the same carcasses but are rarely a focal point of the studies. This study investigated the effects that repeated sampling during experiments have on the decomposition of carrion, measured as both gross biomass (carcass plus fauna) and net biomass (carcass only), on carcasses disturbed on every visit (with weighing only or also with the collection of fauna) and on carcasses disturbed only once. Each trial lasted at least 21 days, with samples taken in triplicate. Rat carcasses used in this study were placed in the field on the same day and either weighed on every visit or ignored until a given day. Internal and ambient air temperatures were recorded on each carcass at the time of sampling and on undisturbed carcasses using temperature loggers. The presence of succession fauna did not result in significant biomass loss on most days; however, there were individual days early in decomposition (days 3 through 6) when the succession fauna comprised a large portion of the gross biomass. With the exception of biomass loss by the emigration of maggots on days 4 and 5, neither repeated weighing of the carcasses nor repeated weighing and faunal sampling of the carcasses statistically affected the rate of biomass loss. Internal temperatures of carcasses sampled repeatedly were frequently 2–5°C lower than those that had not been disturbed, and ambient temperatures differed significantly depending on the location of measurement device. Results indicate that methods used historically for biomass loss determination in experimental forensic entomology studies are adequate, but further refinements to experimental methodology are desirable.

KEYWORDS: forensic science, forensic entomology, carrion, investigator disturbance, biomass loss determination, temperature

Carrion decomposition has been studied primarily in the aspects of forensic entomology/biology (1–4), with the measurement of abiotic parameters, such as biomass loss and temperature, being incidental or only a corollary to the succession patterns of invertebrates on carcasses in experimental studies. Entomological succession and development rate patterns are reputed to be more accurate than some other patterns for the estimation of postmortem intervals, but the effects of the study of succession patterns on biomass loss within experimental investigations have been infrequently studied.

Several published entomological studies have included information on "biomass loss" as a measure of the rate of decomposition (e.g., 5–8). In every case, however, the researchers repeatedly weighed one carcass or a few carcasses at regular, specified times throughout decomposition and succession. In this method, the "carcasses" being weighed included the succession community as well as the carcass itself, resulting in a measure of the gross biomass. Further, carcass and ambient temperature measurement protocols vary widely, ranging from *in situ* thermocouples to distant recording stations. If the techniques used to study entomological patterns have an effect on the rate of biomass loss in experimental carrion, then the affected biomass loss rates could have an effect on the entomological succession patterns. It has already been demonstrated that the carcass environment, including even microenvironments within the carcass, has profound effects on the succession and physiological development of the fauna (8,9). This study is part of a recent effort to examine the effects of investigator disturbance in experimental forensic entomology (10,11).

Putman (12) determined that maggots consumed just over half (532.8 ± 19.2 mg/g original carcass weight) of mouse carcasses in his experiments. Further, calliphorid biomass accounted for 257 ± 10.3 mg/g of the original carcass weight. Because a large proportion of the carcass biomass was being converted to calliphorid tissues, the weight of the carcass when considerable numbers of dipteran larvae are present in experimental carrion decomposition studies could be grossly overstated. This leads to the problem of whether the succession fauna need to be removed from a carcass to obtain an accurate estimate of biomass loss. Alternatively, the faunal biomass is proportionately very small, so that it does not contribute significantly to the overall measure of biomass.

Putman's study (12) was one of two published studies in which the fauna were removed from a carcass during a succession study for the purpose of determining the proportion of gross biomass comprised by the carcass plus the succession fauna. Chaloner et al. (13) examined macroinvertebrate colonization of salmon in Alaskan streams; however, because of the completely different succession community found on carcasses in aquatic situations (14,15) and the aquatic exposure regime, the data from this latter study are not

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Received 12 Aug. 2009; and in revised form 30 Oct. 2009; accepted 1 Nov. 2009.

applicable to terrestrial carrion decomposition. Neither study demonstrated conclusively the influence of fauna on estimates of biomass loss in forensic entomology experiments.

Most carrion research, both in experimental forensic work and for ecological investigations, has followed a simple experimental design. Animal carcasses are used as models for human cadavers, often with a single carcass being sampled frequently and repeatedly over time. Recently, a voluntary protocol has informally emerged for conducting forensic entomology studies (16). Three pig carcasses are used simultaneously for each situation, with different parameters examined on each carcass. One of the carcasses is weighed to determine the rates of biomass loss, the second is used for a qualitative collection of a subsample of arthropods from the carcass to identify succession patterns, and the third is left undisturbed for measurement of a few abiotic parameters such as internal temperatures and for gross visual evaluation of decomposition and succession patterns (16). Sampling protocol on the pig carcasses follows the generally qualitative procedures outlined in Lord and Burger (17) and Haskell and Williams (18) for collection of entomological material. Gross qualitative observations from the third carcass are compared with qualitative samples from the second carcass, generally indicating that the sampling of communities on the second carcass has not had any effect on community composition. However, the effect of this sampling on the rate of biomass loss has not been treated statistically. Further, while one of the carcasses in the three-pig protocol is fitted with a recording thermocouple, it has not been demonstrated whether the repeatedly sampled carcasses experience the same thermal patterns.

Replication within experiments is desired for statistical analysis to examine variability within the data (19,20), but very few current studies have involved rigorous (≥3 samples) replication (e.g., 21–23). Usually in experimental forensic entomology (and even within the voluntary proposed protocol), a single carcass is repeatedly visited and sampled over the course of decomposition and succession. Most studies have used the same carcass for multiple variables, such as biomass loss determination and community succession documentation. This study uses extensive replication in a time-series study on small carcasses with the expectation that invertebrate biomass will influence the observed biomass loss and that the normally rapid decomposition of small carcasses will influence the temperature patterns observed in disturbed versus undisturbed carcasses.

Materials and Methods

Study Site

The study site was located in rural Adams County, Colorado, 19 km north of Denver, located on the west side of Holly Road between Colorado State Highway 7 and Baseline Road. Geographic coordinates for the southwest corner of the field are N39°59′21.8″ W104°55′28.2″. Elevation, from a United States Geological Survey 7.5 min topographic map, was about 1555 m above mean sea level.

The field had been planted with common wheat (*Triticum vulgare* Villars); however, severe crop loss because of persistent drought conditions caused irrigation and harvesting efforts to be abandoned. Other common plant species were field bindweed (*Convolvulus arvensis* L.), ragweed (*Ambrosia artemisifolia* L.), and milkweed (*Asclepias syriaca* L.). To the south of the field was a windbreak of cottonwood trees *(Populus deltoides* Bartram *ex.* Marsh).

Experimental Animals

To obtain fresh carcass tissues, as recommended by Schoenly et al. (24), rats (*Rattus rattus* L.) were purchased alive from Reptilian Haven, Edgewater, Colorado. The rats were free of communicable diseases when purchased. Euthanasia of the rats was conducted on-site using a carbon dioxide–induced hypoxemia in a chamber fashioned from an airtight container fitted to accept carbon dioxide gas from flow-regulated cylinders. Euthanasia was performed immediately prior to the placement of the carcasses. Experimental animal methods were conducted in accordance with the standards of the National Resource Council's Guide to the Care and Use of Laboratory Animals and the Institutional Animal Care and Use Committee rules of the University of Nebraska at Kearney. Rat carcasses were used because the proportion of invertebrate biomass to the total carcass biomass should be more pronounced where there is a high surface-area-to-volume ratio in the host carcass, because the aerobic succession invertebrates are concentrated on exposed surfaces of carcasses. A total of 196 rat carcasses were exposed across two field trials. The rats weighed (mean ± SE) 176.7 ± 2.6 g and 153.2 ± 2.5 g in the first and the second trial, respectively.

Field Methods

This study was conducted during June, July, and August 2002, with the first trial beginning 22 June 2002 and lasting 49 days. The second trial began 20 July 2002 and lasted 21 days. For both trials, the study site was visited and data collections were made every day through day 14, then on days 16, 18, and 21. The first trial was continued, temporally overlapping the second trial, with further visits and data collection on days 28, 32, 35, 38, 41, 44, 46, and 49. Decomposition and succession processes were considered to be complete when the rate of biomass loss slowed to negligible levels (<1% change) for several consecutive visits and when fauna associated with the later decay stages were consistently the only fauna present (16). Although these requirements were met early in the exposure, site visits continued as scheduled.

Each rat carcass was weighed, tagged on a hind leg with a numbered plastic identification tag, and measured for abdominal girth, thoracic girth, and total length minus tail (in mm). Handheld, calibrated spring-type scales accurate to ±1 g were used for weighing the carcasses. Each carcass was laid on a 0.3 m × 0.3 m piece of fiberglass window screen (mesh size *c.* 1 mm) to allow easier collection of the carcass and its succession fauna, but not exclude succession of soil fauna or prevent carcass liquids from leaching into the soil (12). The carcasses were placed in similar attitudes (left side exposed, head pointing north) in a 10 × 13 grid pattern 10 m apart. Although proximity of carcasses to each other may confound some succession patterns (16,25), this layout was chosen for logistical reasons because of the large number of carcasses being used and the small size of the carcasses. Environmental variables (e.g., shading, aspect, slope) can be assumed to be more spatially homogeneous if all the carcasses are in close proximity (26), and spatial autocorrelation was not considered to be a problem because of the random distribution of selected carcasses at the time of collection (27).

On each visit, three carcasses ("DS carcasses") were chosen at random using the randomization function of a handheld calculator, and their attendant fauna were collected. First, aerial adults were netted and preserved separately in 70% ethyl alcohol, because they could possibly be used in further characterization of the carcass community and may aid in the confirmation of larval identifications. Secondly, a representative, qualitative subsample of the

community was collected such that at least one specimen of each taxon in each physiological life stage was collected as identifiable in the field. These organisms were placed directly in glass vials in 70% ethyl alcohol. Thirdly, several live specimens of mature larval Diptera, when present and in addition to the qualitative subsample, were extracted from the community to be weighed and retained alive for rearing to the adult stage, which is often easier to identify. Larval specimens collected for rearing were maintained on 2-day-aged beef liver at room temperature. Biomass of the live organisms was determined upon arrival in the laboratory.

After collection of the community samples, all other physical measurements (internal anal temperature, girths, lengths) were recorded, and the three carcasses plus the remaining succession communities were placed in individual, tared, plastic, zipper-locked bags. Any necrophilic organisms found immediately under the window screening were compiled with the carcass and its attendant fauna under the assumption that they were part of the succession fauna but were confused by the presence of the unnatural screening. The plastic bags containing the carcasses and their succession fauna were weighed and then submerged on-site in a pot of water at ~100°C for 1–2 min to distend and fix Diptera larvae. The contents of the plastic bag were then emptied into a glass Mason jar and preserved with 100% ethyl alcohol.

Nine rat carcasses, chosen at random on the first day of each trial, were weighed in the field (three carcasses) or qualitatively sampled for fauna (three carcasses) or both (three carcasses) on every visit. The methods for weighing or qualitatively sampling the fauna were identical to those used on the destructively sampled (DS) carcasses prior to collection. "W carcasses" were only weighed on every visit, the fauna from the "P carcasses" was sampled qualitatively on every visit (10,11), and the "P&W carcasses" were weighed and qualitatively sampled on every visit. P, W, and P&W carcasses were returned to their exact position on the ground after biomass measurement or collection of insects. Each of these nine carcasses maintained its designation throughout the study.

Following collection, all carcasses were stored in individual glass jars in 100% ethyl alcohol until they could be processed, which involved separation of the succession community from each carcass and biomass determination for each component of carcass and fauna.

Ambient temperatures were measured during the collection of the biotic samples, and internal temperatures were measured at each of the DS, P, W, and P&W carcasses after the collection of biotic samples, but prior to biomass determination. To measure ambient temperatures, a National Institute for Science and Technology (NIST)–certified glass thermometer was held in a shadow and allowed to equilibrate for 5 min, generally while the biotic samples were being collected. To measure the internal temperatures, the thermometer was inserted into the anus of each rat carcass and allowed to equilibrate for 5 min.

Three additional rat carcasses, chosen at random on the first day of each trial, were permanently fitted with a two-channel temperature data logger to record temperatures hourly; these carcasses were not disturbed (weighed or sampled for invertebrates) during the entire course of the study. The body of the logger measured ambient temperatures and was attached to the shaded underside of a board that had been elevated *c.* 7.0 cm above the ground surface using bricks. The top surface of the board was also covered with bricks to prevent the wood from becoming heated and affecting the temperature measurements. A probe extending from the body of the logger was inserted into a rat via the anus to record internal temperatures. The temperature loggers were calibrated against the NIST-certified glass thermometer before and after each field trial. The temperature monitors failed during the first 2 days of exposure in the first trial; therefore, data from those two dates are missing.

Meteorological information, including a general assessment of current climatic conditions and climatic conditions suspected since the previous visit that might impact the succession or decomposition of the carcasses (e.g., rain, high winds), was noted on every visit. Weather data, including daily maximum and minimum air temperatures and precipitation amounts, were retrieved from a certified weather station (28) in Brighton, Colorado, located at N39°59′22″ W104°55′28″, 23.79 km east (bearing 92°) of the study field; additional weather stations were found closer to the study field, but the data were interrupted frequently, so they were deemed to be unusable. Readings from the Brighton weather station were interrupted on days 9–10 of the first trial, so data from these dates are missing.

Statistical Analysis

The gross carcass biomass of a carcass equaled the "biomass" as measured in the field plus the biomass of all the succession fauna samples from that carcass. Net biomass equaled carcass biomass after total succession fauna biomass had been subtracted from the gross biomass. Gross and net biomass measurements for the carcasses were then expressed and analyzed as a percentage of the original carcass biomass. Statistical tests, using NCSS (29), were performed with a 90% significance level ($\alpha = 0.10$) for all analyses (except tests of assumptions, where $\alpha = 0.05$). Student's *t*-tests were used to compare treatments; however, because of the numerous pairwise comparisons, the Bonferroni correction factor (α/k, where k is the number of tests to be performed) was applied when appropriate (20,30). All raw data can be found in De Jong's thesis (10).

- Paired *t*-tests were conducted to determine whether any differences existed between percent gross carcass biomass and percent net carcass biomass at individual sample events. With the Bonferroni correction factor applied, the first trial, with 26 individual tests, and the second trial, with 18 individual tests, were tested at significance levels of $0.10/26 = 0.0038$ and $0.10/18 = 0.0056$, respectively.
- Unpaired *t*-tests were conducted to determine whether any differences existed between percent gross carcass biomass of the DS carcasses and percent gross carcass biomass of the W carcasses for each of the individual site visits. Because of the loss of W carcasses (owing to the overlap of the second trial), this aspect of the study was conducted for only 18 visits in both trials.
- Unpaired *t*-tests were conducted to determine whether any differences existed between percent gross carcass biomass of the DS carcasses and percent gross carcass biomass of the P&W carcasses for each of the individual site visits. The number of individual tests was 26 for the first trial and 18 for the second trial.
- Unpaired *t*-tests were conducted on the data comparing the temperatures measured during collection of the DS carcasses and the temperatures recorded by the temperature loggers during the same hour for both ambient and internal temperatures. Owing to logistic considerations and the random assignment of the carcasses fitted with temperature loggers, the number of individual tests was 18 for both trials.
- Further nonstatistical comparisons were made comparing the temperature data collected during collection of the DS carcasses and the data from the remote weather station in Brighton.

Accumulated degree hours (ADH) were calculated from each set of data (ambient, internal, remote) based on a 0°C threshold using the rectangle method. Missing values were estimated using the difference within the other sets of data. ADH calculations from the first 2 days when the loggers failed were estimated to be the same as the data from the Brighton weather station.

Results

Biomass Loss

Biomass loss over time did not result in a sigmoid curve as described in the literature, instead producing a graph that appeared logarithmic in nature. No statistically significant differences were detected in any of the tests applied in the course of this study regarding loss of biomass, and many percentage differences were observed only in the second decimal place. In the first trial (Table 1), the succession fauna did not make up a substantial proportion of the total biomass as weighed in the field, during either the first 21 days of succession or the first 49 days of succession ($p \geq 0.149$). In the second trial (Table 1), the succession fauna did not make up a significant proportion of the gross biomass ($p \geq 0.036$). However, on days 4 and 5 of succession, third instars of *Sarcophaga* sp. comprised up to 35.3% of the total biomass as weighed in the field, resulting in low *p*-values, but which were not significant with the Bonferroni correction factor applied. On all other dates, *p*-values for gross biomass and net biomass were much higher.

Unpaired *t*-tests conducted on data from the first 21 days of exposure in both trials (Tables 2 and 3) did not reveal any significant differences between the gross biomass of the DS carcasses and field weight of the W carcasses ($p \geq 0.031$). In the second trial (Table 2), there was a large migration of *Sarcophaga* maggots from the W carcasses on the fourth day of succession, evidenced by the reduction of gross biomass of the W carcasses to about 50% of what it had been on the previous day, but the results remained nonsignificant.

TABLE 1—*Percent gross and net biomass remaining (mean ± SE) over time on rat carcasses exposed during summer 2002. Net biomass represents carcass biomass after the removal of succession fauna.* p-*Values are from paired* t-*tests.*

	Trial I (exposed 22 June)			Trial II (exposed 20 July)		
Day	Gross (%)	Net (%)	*p*-value	Gross (%)	Net (%)	*p*-value
0	100 ± 0.0	100 ± 0.0	1.000	100 ± 0.0	100 ± 0.0	1.000
1	90.0 ± 2.2	90.0 ± 2.2	1.000	80.5 ± 3.2	77.7 ± 2.2	0.118
2	75.4 ± 2.3	75.0 ± 2.2	0.149	73.8 ± 2.9	72.3 ± 2.5	0.140
3	68.3 ± 4.2	67.6 ± 4.4	0.199	68.5 ± 8.7	60.2 ± 11.9	0.136
4	59.6 ± 3.1	59.6 ± 3.1	0.184	52.1 ± 6.6	40.2 ± 7.0	0.086
5	59.4 ± 7.1	58.5 ± 7.4	0.220	35.9 ± 8.4	34.4 ± 8.1	0.036
6	48.0 ± 4.5	48.0 ± 4.5	1.000	26.0 ± 3.5	26.0 ± 3.5	0.423
7	33.7 ± 3.4	33.7 ± 3.4	1.000	26.6 ± 1.8	26.5 ± 1.8	0.184
8	35.0 ± 4.2	35.0 ± 4.2	1.000	23.5 ± 1.3	23.5 ± 1.3	1.000
9	31.0 ± 2.9	31.0 ± 2.9	0.423	20.8 ± 2.0	20.8 ± 2.1	1.000
10	26.4 ± 4.7	26.4 ± 4.7	1.000	22.7 ± 3.1	22.6 ± 3.0	0.423
11	27.7 ± 1.8	27.7 ± 1.8	0.423	22.2 ± 1.2	22.2 ± 1.2	1.000
12	26.5 ± 1.7	26.5 ± 1.7	0.423	22.4 ± 2.8	22.2 ± 2.9	0.184
13	29.3 ± 1.8	29.3 ± 1.8	1.000	21.9 ± 1.3	21.9 ± 1.3	1.000
14	28.7 ± 0.8	28.7 ± 0.8	1.000	19.5 ± 1.3	19.5 ± 1.3	0.423
16	23.2 ± 1.0	23.1 ± 0.9	0.270	20.1 ± 1.2	20.1 ± 1.2	1.000
18	26.0 ± 5.1	25.9 ± 5.1	0.423	24.0 ± 4.2	23.9 ± 4.2	0.423
21	26.7 ± 1.2	26.7 ± 1.3	0.423	23.8 ± 2.6	23.8 ± 2.6	0.423

TABLE 2—*Percent gross biomass remaining (mean ± SE) over time between destructively sampled (DS), weighed only (W), and weighed and fauna sampled (P&W) carcasses exposed 22 June 2002.* p-*Values are from* t-*tests comparing data from W and P&W carcasses against data from the DS carcasses.*

Day	DS Carcasses (%)	W Carcasses (%)	*p*-Value	P&W Carcasses (%)	*p*-Value
0	100 ± 0	100 ± 0	1.000	100 ± 0	1.000
1	90 ± 2	91 ± 2	0.874	93 ± 5	0.538
2	75 ± 2	77 ± 3	0.710	78 ± 5	0.691
3	68 ± 4	67 ± 3	0.790	72 ± 3	0.498
4	60 ± 3	57 ± 3	0.544	57 ± 9	0.776
5	59 ± 7	46 ± 4	0.206	45 ± 9	0.305
6	48 ± 4	39 ± 5	0.268	39 ± 7	0.331
7	34 ± 3	36 ± 5	0.706	34 ± 5	0.934
8	35 ± 4	34 ± 4	0.922	32 ± 4	0.675
9	31 ± 3	33 ± 3	0.672	30 ± 3	0.868
10	26 ± 5	30 ± 3	0.534	28 ± 3	0.730
11	28 ± 2	29 ± 2	0.695	27 ± 3	0.946
12	27 ± 2	28 ± 2	0.560	27 ± 3	0.840
13	29 ± 2	28 ± 2	0.563	27 ± 3	0.537
14	29 ± 8	30 ± 4	0.714	29 ± 3	0.984
16	23 ± 10	28 ± 4	0.268	25 ± 3	0.557
18	26 ± 5	36 ± 10	0.406	23 ± 3	0.647
21	27 ± 1	24 ± 1	0.163	23 ± 3	0.412
28	22 ± 3	–	–	22 ± 3	0.936
32	27 ± 2	–	–	24 ± 2	0.373
35	25 ± 2	–	–	24 ± 2	0.723
38	25 ± 1	–	–	26 ± 1	0.455
41	20 ± 1	–	–	26 ± 1	0.013
44	20 ± 2	–	–	27 ± 2	0.059
46	25 ± 2	–	–	26 ± 2	0.801
49	28 ± 3	–	–	23 ± 3	0.282

In the first trial (Table 2), unpaired *t*-tests showed that there were no days in which the gross biomass of the DS carcasses and the field weight of the P&W carcasses were significantly different ($p \geq 0.013$). In the second trial (Table 3), no significant statistical differences were found between gross biomass in the DS carcasses and the gross biomass of the P&W carcasses ($p \geq 0.227$).

Temperature

Ambient temperatures measured hourly by the temperature loggers ranged from 7.0° to 67.4°C in the first trial and from 9.8° to 56.0°C in the second trial, each with an expected diel fluctuation. These data also showed some differences between the instantaneous measurements and contemporaneous readings from the continuous temperature monitors. In the first trial, there were significant differences on days 7, 8, 10, 11, 12, and 14, with higher temperatures recorded by the temperature loggers than measured instantaneously (Table 4). In the second trial, statistically significant differences were found on days 2 and 4, with the temperature logger data higher than the instantaneous measurements only on day 4 (Table 4).

Internal temperatures of undisturbed carcasses ranged from 11.0° to 59.0°C in the first trial and from 8.2° to 62.0°C in the second trial, with a marked diel fluctuation. In the first trial, there were no significant differences ($p \geq 0.05$) between the DS carcass internal temperatures and the monitored carcasses (Table 5). In the second trial, significant differences were found on days 2 and 21 (Table 5), with a higher temperature in the DS carcasses on day 2 but a higher temperature in the monitored carcass on day 21.

Hourly data from the meteorological station in Brighton were less extreme than were recorded by the temperature monitors on-site (Table 4). Temperatures ranged from 11.6° to 34.3°C during the first trial and from 11.4° to 36.7°C during the second trial.

TABLE 3—*Percent gross biomass remaining (mean ± SE) over time from destructively sampled (DS), weighed only (W), and weighed and fauna sampled (P&W) carcasses exposed 20 July 2002.* p-*Values are from* t-*tests comparing data from W and P&W carcasses against data from DS carcasses.*

Day	DS Carcasses (%)	W Carcasses (%)	*p*-Value	P&W Carcasses (%)	*p*-Value
0	100 ± 0	100 ± 0	1.000	100 ± 0	1.000
1	80 ± 3	89 ± 6	0.260	85 ± 2	0.255
2	74 ± 3	75 ± 4	0.838	77 ± 2	0.382
3	68 ± 9	51 ± 8	0.262	61 ± 7	0.592
4	51 ± 7	26 ± 4	0.031	32 ± 5	0.075
5	36 ± 8	24 ± 2	0.253	24 ± 1	0.242
6	26 ± 4	24 ± 3	0.653	24 ± 1	0.621
7	27 ± 2	22 ± 0	0.057	24 ± 1	0.322
8	24 ± 1	21 ± 1	0.166	24 ± 1	0.836
9	21 ± 2	20 ± 1	0.694	24 ± 1	0.281
10	23 ± 3	20 ± 0	0.506	24 ± 2	0.774
11	22 ± 1	21 ± 1	0.560	23 ± 1	0.786
12	22 ± 3	21 ± 1	0.683	24 ± 1	0.613
13	22 ± 1	21 ± 1	0.652	25 ± 1	0.227
14	20 ± 1	21 ± 1	0.520	24 ± 1	0.080
16	20 ± 1	23 ± 1	0.098	25 ± 1	0.030
18	24 ± 4	21 ± 1	0.513	23 ± 1	0.871
21	24 ± 3	20 ± 3	0.447	23 ± 2	0.825

TABLE 5—*Internal temperatures (mean ± SE) from data loggers located on-site and instantaneous measurements during the collection of destructively sampled (DS) carcasses using a glass laboratory thermometer at the time of sampling from rat carcasses exposed during summer 2002.* p-*Values are from unpaired* t-*tests.*

	Trial I (Exposed 22 June)			Trial II (Exposed 20 July)		
Day	Loggers (°C)	DS (°C)	*p*-Value	Loggers (°C)	DS (°C)	*p*-Value
0	n/m*	n/m	–	n/m	n/m	–
1	n/m	54.7 ± 0.3	–	48.3 ± 0.5	44.0 ± 0.0	0.044
2	34.0 ± 2.1	33.7 ± 2.3	0.922	29.9 ± 0.5	33.3 ± 0.3	0.004
3	31.3 ± 0.8	31.0 ± 2.1	0.913	38.2 ± 0.4	40.3 ± 1.2	0.163
4	39.0 ± 1.8	40.0 ± 2.1	0.728	42.3 ± 0.3	42.0 ± 0.0	0.508
5	35.3 ± 1.1	34.3 ± 2.0	0.698	30.2 ± 0.7	31.7 ± 0.3	0.143
6	37.6 ± 0.8	38.0 ± 0.0	0.628	40.8 ± 0.0	n/m	–
7	53.2 ± 1.7	47.3 ± 0.9	0.038	38.6 ± 0.0	n/m	–
8	50.5 ± 1.0	45.3 ± 1.9	0.070	34.9 ± 1.0	31.0 ± 0.0	0.056
9	48.5 ± 1.7	44.0 ± 2.3	0.185	40.7 ± 0.2	41.0 ± 0.0	0.286
10	45.3 ± 1.9	42.7 ± 0.7	0.257	40.9 ± 0.7	40.7 ± 0.3	0.769
11	45.1 ± 0.4	42.5 ± 0.5	0.033	40.4 ± 0.3	40.5 ± 0.5	0.915
12	49.3 ± 1.8	45.3 ± 1.8	0.197	26.1 ± 0.1	24.5 ± 0.5	0.031
13	41.9 ± 1.0	42.7 ± 0.7	0.544	35.9 ± 0.0	n/m	–
14	46.3 ± 1.7	42.0 ± 0.6	0.075	54.0 ± 0.0	n/m	–
16	37.4 ± 0.7	35.0 ± 0.6	0.051	19.6 ± 0.0	n/m	–
18	21.3 ± 0.2	19.5 ± 1.5	0.211	33.2 ± 1.7	26.0 ± 0.0	0.161
21	46.4 ± 0.0	n/m	–	49.8 ± 1.3	33.0 ± 1.0	0.003

*n/m = not measured because of equipment failure.

Discussion

The logarithmic nature of the decomposition appears to be a result of the timing of visits. The second visit was 1 day after placement of the carcasses, but the carcasses had already started to undergo rapid decomposition. Rapid decomposition likely eliminated the initial horizontal asymptote on a normal decomposition curve as observed in other warm climates (16,25).

The results from this experiment indicate that investigator disturbance does not have a significant impact on the overall rate of biomass loss when sampling repeatedly on rat carcasses. There are, however, specific periods of time during active decomposition (such as days 3–6, when biomass loss was greatest) in which investigator disturbance may have a noticeable effect on the rate of biomass loss. From the perspective of strict forensic entomology (looking only at the succession fauna), this should not invalidate previous studies of carrion succession as the entomological aspects did not appear to be affected considerably by the altered rate of decomposition (10,11).

Although there was little statistical significance between the temperature data from the loggers and the instantaneous measurements during the collection of DS carcasses, there were noticeable discrepancies. These frequent discrepancies are probably best explained by slightly different methods and the effect of microenvironments. For the carcasses with temperature loggers, the temperature measured was shaded *c.* 7.0 cm above the ground surface, and

TABLE 4—*Ambient temperatures (mean ± SE) from data loggers located on-site, instantaneous measurements during the collection of destructively sampled (DS) carcasses using a glass laboratory thermometer, and one remote weather station at the time of sampling from rat carcasses exposed during summer 2002.* p-*Values are from unpaired* t-*tests comparing data between data loggers and spot measurements.*

	Trial I (Exposed 22 June)				Trial II (Exposed 20 July)			
Day	Loggers (°C)	DS (°C)	*p*-Value	Remote (°C)	Loggers (°C)	DS (°C)	*p*-Value	Remote (°C)
0	n/m*	n/m	–	32.1	n/m	31.0 ± 0.0	–	30.1
1	n/m	38.3 ± 0.3	–	31.7	38.6 ± 1.0	37.0 ± 0.0	0.511	27.5
2	29.7 ± 0.6	29.0 ± 2.1	0.813	31.4	27.7 ± 0.1	31.0 ± 0.0	<0.001	25.6
3	28.2 ± 0.5	26.0 ± 0.0	0.010	23.0	34.6 ± 0.3	33.7 ± 0.7	0.280	28.6
4	34.3 ± 1.2	32.3 ± 0.3	0.187	31.1	42.2 ± 0.4	36.3 ± 0.3	<0.001	35.2
5	32.9 ± 0.5	32.0 ± 0.0	0.146	31.3	27.3 ± 0.5	28.3 ± 0.3	0.136	25.8
6	35.6 ± 1.0	33.7 ± 0.3	0.142	29.6	37.2 ± 0.0	n/m	–	32.3
7	53.0 ± 1.2	39.3 ± 0.3	<0.001	34.9	24.7 ± 0.0	n/m	–	31.2
8	49.7 ± 0.2	35.0 ± 0.0	<0.001	31.9	30.3 ± 1.4	29.5 ± 0.5	0.691	28.8
9	46.9 ± 1.3	39.0 ± 0.0	0.004	n/m	38.6 ± 0.4	37.5 ± 0.5	0.173	33.9
10	43.3 ± 1.1	33.7 ± 0.3	0.001	n/m	37.9 ± 0.9	36.7 ± 0.3	0.278	35.1
11	42.2 ± 0.8	35.7 ± 0.3	0.002	30.3	38.8 ± 0.7	37.0 ± 1.0	0.221	36.3
12	46.9 ± 0.9	35.3 ± 0.3	<0.001	30.4	23.0 ± 0.3	24.5 ± 0.5	0.055	20.9
13	37.6 ± 0.8	34.3 ± 0.3	0.018	29.8	32.8 ± 0.0	n/m	–	30.2
14	46.1 ± 1.6	35.0 ± 0.0	0.002	25.8	39.3 ± 0.0	n/m	–	28.6
16	35.2 ± 1.4	31.0 ± 0.0	0.037	31.8	18.7 ± 0.0	n/m	–	20.8
18	16.6 ± 0.3	20.7 ± 2.7	0.207	23.7	31.0 ± 1.4	25.0 ± 0.0	0.159	25.8
21	47.9 ± 0.0	n/m	–	27.9	36.4 ± 0.9	32.7 ± 0.3	0.016	29.0

*n/m = not measured because of equipment failure.

probably was more reflective of ground surface temperatures than of ambient air temperatures. When an instantaneous temperature was taken in the field, the glass thermometer was held in a shadow about 30 cm from the ground surface. These differences testify to the particular sensitivity of microclimatic conditions (7 vs. 30 cm above the ground), which should be anticipated and measured appropriately in carrion decomposition studies (31).

Internal temperatures have a profound effect on the metabolic growth of succession fauna, even to the extent of altering succession patterns (9). Because of the small size of the carcasses, very large aggregations of dipteran larvae were not produced, so internal temperatures, although higher, tracked ambient air temperatures, especially at night. Further, because the larval Diptera aggregations did not achieve large sizes nor do they remain present for a long time, the metabolic heat generated did not exert a large influence on the internal temperatures (cf. [9,32]).

Instead, it appears that the internal temperatures were probably more influenced by ground temperatures and the insulation provided by the mummified skin of the rat carcasses. No carcasses in the field were exposed to shaded conditions; thus, they experienced both solar radiation heat and residual ground heat. Ground reflectance in agricultural fields has been shown to be very high (33), and the increased albedo may have caused the ground temperatures to be considerably higher than the air temperatures.

The consistently lower internal temperatures in the DS carcasses in comparison to the carcasses with temperature loggers may be because of the measurement of the DS carcasses after minor disturbances. Aerial adult insects and a representative community sample had been collected before the internal temperatures were taken. These minor disturbances might be responsible for statistically significant lower temperatures than those recorded from the entirely undisturbed carcasses with temperature loggers, and these data should be considered in future forensic entomology experiments and casework.

The lower nighttime temperatures recorded by the temperature monitors on-site than those from the remote meteorological station were probably a result of higher nighttime humidity near the ground surface where the loggers were located (33). Higher daytime temperatures in the field were most likely a result of measuring ground temperatures as opposed to air temperatures, as discussed previously. Goff (16) describes the use of regressions of data from remote weather stations to that collected from an on-site station for calibration of temperature data.

Calculation of ADH using the different measurement methods provided widely disparate results. *Sarcophaga* sp. maggots were generally present from the third to the seventh days of exposure (10,11), and ADH estimates by day seven varied by 1071 ADH between the three measurement methods (Table 3). These differences of ≤1071 ADH could potentially make a significant difference in the estimation of postmortem intervals when applied to organismal development rates.

While the results found in this study appear to validate the methods used historically, care should still be taken to determine that experimental data are collected appropriately. Decomposition rates of rodent carcasses are vastly different when exposed to alternate environments, such as temperate forests (34–37), and are likely different from decomposition rates in larger carcasses. This study was conducted with small mammal carcasses (<210 g) because it was expected that significant results would be observed more readily in host carrion with a large surface-area-to-volume ratio. Because the aerobic requirements of the arthropod succession fauna restrict it to the surface of the carrion, we anticipate that host carcasses with lower surface area to volume ratios (such as pigs or other human-sized carcasses) should have proportionately smaller succession fauna communities, affecting the rates of biomass loss even less than what was measured in this study. However, further research investigating the validity of current experimental techniques could involve larger carcasses (particularly pigs for standardization with regular protocols and comparability to humans) sampled more intensively during active decomposition stages, which showed slightly significant deviations in this study.

The temperature aspects of this study illustrate the importance of using appropriate controlled techniques in experimental forensic entomology. Minor disturbance of the rat carcasses during sampling generally produced lower recorded internal temperatures than in those that had no disturbance whatsoever, demonstrating the sensitivity of the carrion ecosystem to disturbance. The effects of such slight differences on the succession pattern are not yet known, but may be cumulative, and could involve overestimation of the postmortem interval if based on lower measured internal temperatures.

Acknowledgments

We thank the following for their participation in this project: Rev. Bart Janz and the congregation of Holly Ridge Baptist Church graciously allowed use of their land. Douglas De Jong provided technical assistance in the field. Jim Chadwick and John Foster provided advice on the project in its initial and final stages, respectively. Two anonymous reviewers provided useful comments on the manuscript prior to publication. The work described herein was conducted in partial fulfillment of the requirements for the degree of Master of Science by GDD through the University of Nebraska-Lincoln, advised by WWH, LGH, and John Foster.

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