Effect of Investigator Disturbance in Experimental Forensic Entomology: Succession and Community Composition

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Abstract
Carrion insect succession studies have historically used repeated sampling of single or a few carcasses to produce data, either weighing the carcasses, removing a qualitative subsample of the fauna present, or both, on every visit over the course of decomposition and succession. This study, conducted in a set of related experimental hypotheses with two trials in a single season, investigated the effect that repeated sampling has on insect succession, determined by the number of taxa collected on each visit and by community composition. Each trial lasted at least 21 days, with daily visits on the first 14 days. Rat carcasses used in this study were all placed in the field on the same day, but then either sampled qualitatively on every visit (similar to most succession studies) or ignored until a given day of succession, when they were sampled qualitatively (a subsample) and then destructively sampled in their entirety. Carcasses sampled on every visit were in two groups: those from which only a sample of the fauna was taken and those from which a sample of fauna was taken and the carcass was weighed for biomass determination. Of the carcasses visited only once, the number of taxa in subsamples was compared to the actual number of taxa present when the carcass was destructively sampled to determine if the subsamples adequately represented the total carcass fauna. Data from the qualitative subsamples of those carcasses visited only once were also compared to data collected from carcasses that were sampled on every visit to investigate the effect of the repeated sampling. A total of 39 taxa were collected from carcasses during the study and the component taxa...
are discussed individually in relation to their role in succession. Number of taxa differed on only one visit between the qualitative subsamples and the actual number of taxa present, primarily because the organisms missed by the qualitative sampling were cryptic (hidden deep within body cavities) or rare (only represented by very few specimens). There were no differences discovered between number of taxa in qualitative subsamples from carcasses sampled repeatedly (with or without biomass determinations) and those sampled only a single time. Community composition differed considerably in later stages of decomposition, with disparate communities due primarily to small numbers of rare taxa. These results indicate that the methods used historically for community composition determination in experimental forensic entomology are generally adequate.

**Keywords:** Dermestidae, Formicidae, Sarcophagidae, carrion, community composition, forensic entomology, investigator disturbance, succession

**Introduction**

Carrion research has recently received much scientific inquiry due to its application in the medico-legal sciences as forensic entomology/biology (Erzinçlioğlu, 1983; Keh, 1985; Byrd & Castner, 2000; Benecke, 2001). The primary use of entomology in the forensic context concerns the examination of succession patterns and developmental rates of succession fauna for estimation of postmortem interval and, consequently, minimum time since death. This application, among others such as toxicology, distributional analysis to place a suspect/victim at the scene of a crime, identification of serological evidence through gut analyses, etc., has led to both conviction and exoneration of suspects in human (homicide, suicide, accidental death) and nonhuman (poaching) deaths (e.g. Greenberg, 1985; Lord, 1990; Benecke, 1998; Introna et al., 1998, 2001).

Most carrion research studies, both in experimental forensic work and for ecological investigations, have followed a simple experimental design. Animal carcasses are used as models for human cadavers because of prohibitive legislation regarding the disposal and research uses of the latter. Experimental forensic entomology studies have generally focused on the areas of biomass loss as a measure of decomposition, succession fauna community dynamics, and effects of abiotic parameters such as temperature. This study examines assumptions of experimental forensic entomology and focuses on the impact of investigator disturbance on the dynamics of the succession community, conducted in conjunction with other studies on the rate of biomass loss (De Jong, 2003).

Of considerable interest to both general ecology and forensic science is the community composition and its changes over time during succession on carrion. Most experimental work to date has involved repeatedly visiting the same carcass(es) over time and removing a qualitative sample of the fauna present at each visit for identification. Unfortunately, such qualitative sampling may disrupt the community and can only provide presence/absence information with a subjective opinion of abundance. Although researchers have been concerned with potential effects of repeated disturbance, few studies have rigorously investigated whether the repeated sampling in these studies alters succession patterns or whether the samples obtained are truly representative of the actual community present.

Concerns over the use of a single carcass for both biomass loss determination and collection of community samples have led some researchers to adopt a voluntary protocol
using three pig carcasses. The carcasses are used simultaneously, with different parameters examined on each carcass. One of the carcasses is weighed to determine rates of biomass loss, the second is used for a qualitative collection of a sample 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 (Goff, 2000). Sampling protocol on the pig carcasses follows the generally qualitative procedures outlined in Lord & Burger (1983) and Haskell & Williams (1990) for collection of entomological material. The gross qualitative observations from the third carcass are compared with the qualitative samples from the second carcass, generally indicating that the sampling of communities on the second carcass had no noticeable effect on community composition. However, the effect of this sampling has not been treated statistically and the faunal samples remain only qualitative. Questions of whether or not the community in the samples is representative of the actual community present and whether the repeated sampling affects subsequent community composition and succession patterns remain unanswered.

To avoid the potential problem of disturbance of natural succession through invasive qualitative sampling, some experimental forensic entomology studies have made use of carrion-baited traps, interception traps or, very rarely, destructive sampling. Carrion baited traps provide quantitative data but may disrupt the normal emigration patterns, giving a skewed picture of succession pattern; these have primarily been used in the study of spatial community composition of particular taxonomic groups [e.g. Silphidae (Shubec, 1983, 1984), Calliphoridae and other filth flies (Burger, 1965; Erzinçlioğlu, 1980)] rather than for the strict study of carrion succession. It is also possible that the succession of a later-arriving species might be predicated upon carcass preparation or emigration by an early arriving species. Interception traps also provide quantitative data and arrival sequence patterns with correlation to different stages of carrion decomposition (Kentner & Streit, 1990), but only sample the fauna as it approaches or leaves the carrion, allowing only an inferred estimate as to the actual community present. The organisms collected in the ingress portion of these traps may misrepresent the actual community utilizing the carcasses (De Jong, 1993).

A complete, destructive census irreparably disrupts or ends the succession but can provide comprehensive data (De Jong & Chadwick, 1997; Tomberlin & Adler, 1998; Chaloner et al., 2002). However, for the scope of most forensic entomology experiments, destructive sampling is not a viable research strategy. In this study, we used replicated, quantitative samples and destructive sampling techniques in a time-series study to statistically investigate the effects of repeated investigator disturbance on the succession community and succession patterns.

Materials and methods

Study site
The study site was located in rural Adams County, Colorado, USA, 19 km north of Denver, on the west side of Holly Road between Colorado State Highway 7 and Baseline Road. Geographical coordinates for the southwest corner of the field are 39°59’21.8”N,
104°55'28.2"W. Elevation, from a United States Geological Survey 7.5-minute topographic map, was about 1555 m above mean sea level.

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

**Experimental animals**

To obtain fresh carcass tissues (Schoenly et al., 1991), rats (*Rattus rattus* Linnaeus) were purchased alive from Reptilian Haven, Edgewater, Colorado, and were free of communicable diseases when purchased. A total of 196 rat carcasses was used across two field trials. The rats weighed (mean ± SE) 176.7 ± 2.6 g in the first trial and 153.2 ± 2.5 g in the second trial. Euthanasia of the rats was conducted onsite using a CO₂-induced hypoxemia in an airtight container fitted to accept CO₂ gas from flow-regulated cylinders. Euthanasia occurred at the site immediately prior to placement of the carcasses.

**Field methods**

This study took place during June, July, and August 2002, with the first trial beginning 22 June 2002 and a second trial beginning 20 July 2002. Each trial lasted at least 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 between visits), remaining in that situation for several consecutive visits, and when fauna associated with the later decay stages (Goff, 2000) were consistently the only fauna present. Although these requirements were met early in the exposure, site visits continued according to the above schedule.

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 × 0.3 m piece of fiberglass window screen (mesh size approximately 1 mm) to allow for easier collection of the carcass and its succession fauna but not to exclude succession of soil fauna or prevent carcass liquids from leaching into the soil (Putman, 1978b). 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 (Tomberlin & Adler, 1998; Goff, 2000), 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, etc.) can be assumed to be more spatially homogeneous if all the carcasses are in close proximity (Dutilleul, 1993), and spatial autocorrelation was not considered to be a problem due to the random distribution of carcass conditions at time of collection (Legendre, 1993).
On each visit, three randomly chosen carcasses (“DS carcasses,” so named due to being destructively sampled) and their attendant faunas were collected in their entirety. First, aerial adults that were present on the carcasses were netted and preserved separately in 70% ethyl alcohol because they could potentially be used in further characterization of the carcass community (cf. De Jong & Chadwick, 1997) and aid in confirmation of larval identifications. Adult Diptera collected in the aerial nets were not counted, because they were not sampled quantitatively. Second, 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. Third, 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 taxonomically. 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, and 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 screen. The plastic bags containing the carcasses and their succession fauna were weighed and then submerged onsite in a pot of water at ~100°C for 1–2 min to distend and fix Diptera larvae. Contents of the plastic bag were then emptied into a glass Mason jar and preserved with 100% ethyl alcohol until they could be processed, which involved separation of the remainder of the succession community from each carcass.

Nine rat carcasses, chosen at random on the first day of each trial, were weighed in the field (three “W carcasses”) or qualitatively sampled for fauna (three “P carcasses”) or both (three “P & W carcasses”) on every visit. The methods for weighing or qualitatively sampling the fauna were identical to those used on the DS carcasses prior to collection. “W carcasses” were only weighed on every visit (data regarding these are analyzed in De Jong 2003), the fauna from the “P carcasses” was sampled qualitatively on every visit 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 (Goff, 2000). Each of these nine carcasses maintained its designation throughout the study.

The members of the communities from all samples were sorted, identified to physiological life stage (egg, larva, pupa, or adult, with stage determination for larval Diptera and sex determination for adult Diptera) and lowest practical taxonomic level using available keys, and counted. For taxa normally associated with carrion in the literature, identifications were made to the genus or species level, except early stage larvae and larval Sarcophagidae, which were identified only to the family level.
Environmental variables
Three additional rat carcasses, chosen at random on the first day of each trial, were perma-
nently fitted with a two-channel temperature data logger to record temperatures hourly, but
these carcasses were not weighed or qualitatively sampled for invertebrates. The
shaded body of the logger measured ambient temperatures, and a probe was inserted into
a rat via the anus to record internal temperatures. Meteorological notes, including a gen-
eral 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, etc.), were made on every visit. Weather data, including daily maximum and
minimum air temperatures and precipitation amounts, were also retrieved from a certified
weather station in Brighton, Colorado, located at 39°59′22″N, 104°55′28″W, 23.79 km east
(bearing 92°) of the study field (http://www.weatherunderground.com). Data are reported
in De Jong (2003).

Statistical analyses
Number of taxa was the primary variable analyzed using statistical tests, as a general
measure of the community; however, community similarity indices were also used to fur-
ther refine the analyses. Statistical tests, using NCSS (Hintze, 2001), were performed with
a 90% significance level (α = 0.10) for all analyses (except tests of assumptions, where α =
0.05). Students t-tests were used to compare treatments; however, because of the numerous
pairwise comparisons, the Bonferroni correction factor (α/k, where k = number of tests to
be performed) was applied when appropriate (Sokal & Rohlf, 1995; Zar, 1999). All raw data
can be found in De Jong (2003).

Paired t-tests were conducted to determine if any differences existed between the num-
ber of taxa collected in samples from the DS carcasses and the total number of taxa actually
present. 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. The proportion of taxa collected in the
samples to the total taxa collected was calculated as a measure of the amount of the com-

Unpaired t-tests were conducted to determine if any differences existed between num-
ber of taxa collected in samples from the DS carcasses and the number of taxa collected
from the P carcasses for each of the individual site visits. This aspect of the study was
conducted for 26 visits in the first trial and 18 visits in the second trial. The Jaccard Com-
munity Similarity Index was calculated for the taxa present on each visit from the two
treatments to determine if differences might exist between the composition of the commu-
nities in the two treatments.

Unpaired t-tests were conducted to determine if any differences existed between num-
ber of taxa collected in samples from the DS carcasses and the number of taxa collected
from 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. The Jaccard Community Similarity
Index was also conducted for this comparison.
Results and discussion

Succession

Studies that involve destructive sampling for the purpose of ascertaining the succession community are few (Putman, 1978a,b; Kuusela & Hanski, 1982; Isiche et al., 1992; Tomberlin & Adler, 1998) and are generally concerned with only one or a few taxonomic groups. For each of these studies, although other succession fauna may have been present, only Diptera, Coleoptera, and a few other data were published. De Jong & Chadwick (1997) and Chaloner et al. (2002) reported a numerical census of all taxa collected in each of their studies.

Over the course of two trials in the present study, 38 distinct arthropod taxa were collected from 196 rat carcasses (Table 1). Of these, 18 taxa are generally recognized as necrophilic. The other taxa were considered to be “incidental.” These incidental taxa included all Heteroptera and Lepidoptera, Coccinella novemnotata Herbst (Coleoptera: Coccinellidae), Elodes sp. (Coleoptera: Tenebrionidae), unidentified Anthicidae, Carabidae, Elateridae, and Tenebrionidae (all Coleoptera), and an unidentified Otididae (Diptera). Four non-insect taxa were also collected, including mesostigmatid mites in the genus Poecilochirus, a few specimens of the prostigmatid mite family Anystidae and one specimen each of an unidentified eremaeid mite nymph (Acarina: Oribatida) and the spider Talavera minuta (Banks) (Araneida: Salticidae). The incidental taxa and all non-insect arthropods were excluded from the statistical analyses. Because of their brief temporal association with a given carcass, adult Diptera were also excluded from the analyses; therefore, only 13 taxa were included. The total number of taxa and number of necrophilic taxa collected from these rat carcasses was comparable to that found in other studies in Colorado and other arid/semiarid regions of North America (Burger, 1965; McKinnerney, 1978; Schoenly, 1981; Schoenly & Reid, 1983; France et al., 1992, 1997; De Jong, 1993; De Jong & Chadwick, 1997, 1999).

Many calliphorid eggs and a few first-stage larvae of Calliphoridae were found on carcasses in the early stages of decomposition. In only one carcass were second- and third-stage larvae of the calliphorid Cochliomyia macellaria (Fabricius) collected. As adults, C. macellaria were collected infrequently, whereas adults of Phormia regina (Meigen) and Lucilia sericata Meigen were more common. Calliphora coloradensis Hough, Calliphora livida Hall, and Calliphora vicina Robineau-Desvoidy adults were also collected in small numbers. No calliphorid adults were collected on any of the carcasses after day 12 of exposure. All six taxa are common calliphorids in Colorado, with C. macellaria and L. sericata usually particularly common in the Plains region (De Jong, 1994; De Jong & Chadwick, 1997).
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<th>INSECTA</th>
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<tr>
<td>Hemiptera</td>
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<td>Lygaeidae</td>
<td>Calliphoridae</td>
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<td>Lygaeus kalmii (Say)</td>
<td>Phormia regina Say</td>
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<td>Unidentified Lygaeidae</td>
<td>Calliphora coloradensis Hough</td>
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<td>Coleoptera</td>
<td>Calliphora livida Hall</td>
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<td>Carabidae</td>
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<td>Unidentified Carabidae</td>
<td>Cochliomyia macellaria (F.)</td>
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<td>Histeridae</td>
<td>Lucilia sericata Meigen</td>
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<td>Saprinus sp.</td>
<td>Unidentified Calliphoridae (larvae)</td>
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<td>Coleoptera (cont.)</td>
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<td>Histeridae (larvae)</td>
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<td>Coleoptera</td>
<td>?Dexosarcophaga transita Townsend</td>
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<td>Coccinellidae</td>
<td>Espyphotonima sp.</td>
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<td>Coccinella novemnotata Herbst</td>
<td>Linsarcophaga sp.</td>
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<td>Unidentified Anthicidae</td>
<td>Sarcodexia sp.</td>
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<td>Elateridae</td>
<td>Sarcophaga bullata Parker</td>
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<td>Unidentified Elateridae</td>
<td>Unidentified Sarcophagidae (larvae)</td>
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<td>Dermentidae</td>
<td>Scathophagida</td>
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<td>Dermentes sp. (larvae)</td>
<td>Scathophaga stercoraria (L.)</td>
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<td>Dermentes frischii Kügelann</td>
<td>Sepsida</td>
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<td>Dermentes marmoratus Say</td>
<td>Themira putris (L.)</td>
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<td>Tenebrionidae</td>
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<td>Elodes sp.</td>
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<td>Unidentified Tenebrionidae</td>
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<tr>
<td>Nitidulidae</td>
<td>Hymenoptera</td>
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<td>Nitidula ziczac Say</td>
<td>Formicidae</td>
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<td>Omosita color (L.)</td>
<td>Lasius alienus Provancher</td>
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<td>Cleridae</td>
<td>Myrmica sp.</td>
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<td>Neerobia rufipes (De Geer)</td>
<td>ACARI</td>
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<td>Silphidae</td>
<td>Mesostigmata</td>
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<td>Thamathorphilus lapponicus Herbst</td>
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<td>Staphyllinidae</td>
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<td>Noctuidae</td>
<td>Labidognatha</td>
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<td>Unidentified Noctuidae</td>
<td>Salticidae</td>
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<td>Talarva minuta (Banks)</td>
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Although five distinct taxa of Sarcophagidae adults were present at the carcasses, only one species was reared from collected larvae. The emerged adults were identified as Sarcophaga bullata Parker; however, due to very similar morphologies of species in the genus (Wells et al., 2001), the taxon, especially in larval habitus, is referred to as Sarcophaga sp. In both trials, Sarcophaga maggots dominated the community numerically and in terms of biomass early in succession, then completed their development and migrated to soils under or near the carcasses for pupation by day 6. The other sarcophagid taxa collected (all as adults) were identified as Dexosarcophaga transita Townsend, Liosarcophaga sp., Neobellieria sp. and Sarcodexia sp. Sarcophagid adults were present on carcasses through the ninth day of exposure in both trials.
It is noteworthy that, even though several calliphorid and sarcophagid species were present at the study sites as adults, only one sarcophagid species was extremely successful in colonizing the carcasses. Viviparity/ovoviviparity, a trait ubiquitous in the Sarcophagidae (Shewell, 1987b), can occasionally be a more competitive life history trait than oviparity, which is the most common trait in the Calliphoridae (Shewell, 1987a), especially in small carcasses near the Front Range of Colorado (G. D. De Jong, personal observation). This may have led to the success of the sarcophagids over the calliphorids in this study. The *Sarcophaga* species was by far the most abundant sarcophagid species present, which probably accounted for its success over the other sarcophagids.

Other necrophagous Diptera families included the Scathophagidae and Sepsidae. Each was represented by a single taxon, *Scathophaga stercoraria* (Linnaeus) and *Themira putris* (Linnaeus), respectively. One adult of each taxon was collected during succession—*S. stercoraria* on day 6 and *T. putris* on day 7 of the second trial.

Necrophilous Coleoptera were represented by taxa in six families. Two dermestid species were collected, with *Dermestes frischii* Kügelann usually occurring slightly (3–7 days) earlier in succession than *Dermestes marmoratus* Say. Both species, however, continued to be present sporadically throughout succession, with *D. frischii* more common. One of these species, *D. frischii*, is sometimes a dominant beetle at carrion, especially in later stages of decomposition (Hegazi et al., 1991). *Dermestes marmoratus* seems to be more sporadic in its presence at carrion, appearing in some studies and not in others conducted in the same vicinity using similar carrion (Schoenly, 1981). Larvae of *Dermestes* were infrequently collected in this study. In Colorado, *Dermestes caninus* Germar, *D. frischii*, and *D. marmoratus* are the only dermestid species that have previously been reported in carrion decomposition studies (De Jong, 1993; De Jong & Chadwick, 1997, 1999), usually arriving on carcasses after some decomposition, dehydration, and/or desiccation of tissues has occurred, indicating that these results are not unusual.

Ten adult specimens of the histerid beetle *Saprinus* sp. were collected, as well as two unidentified larval histerids. Histerid beetles are voracious predators of dipteran larvae (Nuorteva, 1970) and silphid beetles (W. W. Hoback, personal observation) and have been used as potential biological controls for muscid larvae on farms and in slaughterhouses. The histerids collected in this study were present only through day 6 of succession, during the period of time in which *Sarcophaga* sp. and *C. macellaria* were present on carcasses, suggesting that their presence was likely for predation on the maggots.

Three specimens each of *Nitidula ziczac* Say and *Omosita colon* (Linnaeus) were collected over the course of the two trials. *Nitidula ziczac* was collected during the first 5 days of succession, whereas all three specimens of *O. colon* were collected on day 18. All six nitidulids were collected on P carcasses, but due to the low densities it is not possible to determine if their presence/absence was because of treatment effects. Nitidulids are common saprophagous organisms (Parsons, 1943), but only the genera *Carpophilus*, *Glischrochirus*, *Nitidula*, and *Omosita* have been observed to frequent carrion in Colorado (Adair & Kondratieff, 1996; De Jong & Chadwick, 1997, 1999; G. D. De Jong, personal observation). A single specimen of a clerid, the red-legged ham beetle, *Necrobia rufipes* (De Geer), was collected on the final day of the study on a DS carcass.
Although silphid beetles of the genus *Nicrophorus* are frequently associated with small carcasses, especially preferring those of sizes similar to these rat carcasses (Scott, 1998), *Nicrophorus* beetles were not observed or collected frequently in this study. Only one specimen of *N. marginatus* Fabricius, a common species in North America (Peck & Kaulbars, 1987; Lingafelter, 1995; Ratcliffe, 1996), was collected during sampling (P & W carcass, Trial I, day 2). Three other specimens of *Nicrophorus* were incidentally observed at rat carcasses in the field; however, those specimens were not collected because the particular carcasses on which they were observed had not been randomly selected for collection or disturbance on those days. All four specimens were observed or collected within the first 3 days of succession. Drought conditions experienced throughout the central United States in 2002 appear to have reduced populations of *Nicrophorus* regionally (Bedick et al. 2006). One other species of silphid beetle, *Thanatophilus lapponicus* Herbst, was represented by three specimens collected only within the first 2 days of succession.

The only staphylinid beetle collected was *Creophilus maxillosus* (Linnaeus). Four specimens of this predaceous and saprophagous species were collected during the succession, only in company with *Sarcophaga* sp. Kramer (1955) stressed the importance of humidity in the rearing of *C. maxillosus*, and the extreme desiccation of the carcasses may have prevented their further occurrence. *Creophilus maxillosus* is generally common on carcasses in Colorado (De Jong, 1993; De Jong & Chadwick, 1997, 1999).

Nests of the ant *Myrmica* sp. were observed throughout the wheat field, and carcasses that were incidentally placed near the nests were often overrun with ants. Although only observed subjectively, inundations of ants on the carcasses appeared to result in reductions in dipteran colonization. Ant predation on eggs and first-stage larvae of calliphorids and first-stage larvae of sarcophagids appeared to be responsible for the reduced dipteran involvement in succession on those carcasses. Larger dipteran larvae appeared not to have been affected as much as earlier larval stages and co-occurred with *Myrmica* sp., if established before predation on the eggs or first-stage larvae by ants. Similar depredations by ants on succession fauna have been reported in the literature (Wells & Greenberg, 1994; Stoker et al., 1995). Only one other species of ant, *Lasius alienus*, was found, and this taxon was represented by a single specimen.

**Representability of qualitative samples**

Only one statistically significant difference was found between the number of taxa in samples from the DS carcasses and the number of taxa actually present (Table 2, $P = 0.001$): on the first day of the second trial, *Sarcophaga* sp. was the only taxon (1 ± 0 taxa) collected in each of the three replicate samples; however, after processing it was discovered that each carcass actually had three taxa (3 ± 0 taxa) present in various combinations of *Sarcophaga* sp., *Saprinus* sp., *T. lapponicus*, *C. maxillosus*, and *Myrmica* sp. With this exception and correcting for multiple comparisons ($\alpha = 0.0038$ and $\alpha = 0.0056$ in the first and second trials, respectively), the number of taxa collected in samples from the DS carcasses was not significantly different from the total number of taxa present at the carcasses ($P \geq 0.038$).
Table 2. Number of taxa (mean ± SE) in field-collected qualitative samples and total number of taxa (mean ± SE) present on “destructively sampled (DS)” rat carcasses exposed during summer 2002. *P*-values are from paired *t*-tests, *n* = 3.

<table>
<thead>
<tr>
<th>Day</th>
<th>Trial I (exposed 22 June)</th>
<th>Trial II (exposed 20 July)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample Total P-value</td>
<td>Sample Total P-value</td>
</tr>
<tr>
<td>0</td>
<td>0.0 ± 0.0 0.0 ± 0.0 1.000</td>
<td>0.0 ± 0.0 0.0 ± 0.0 1.000</td>
</tr>
<tr>
<td>1</td>
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<td>1.0 ± 0.0 3.0 ± 0.0 0.000</td>
</tr>
<tr>
<td>2</td>
<td>1.7 ± 0.3 2.7 ± 0.7 0.423</td>
<td>2.0 ± 0.0 2.0 ± 0.0 1.000</td>
</tr>
<tr>
<td>3</td>
<td>1.0 ± 0.0 2.3 ± 0.3 0.057</td>
<td>1.7 ± 0.7 3.3 ± 0.9 0.038</td>
</tr>
<tr>
<td>4</td>
<td>1.0 ± 0.6 1.0 ± 0.6 1.000</td>
<td>2.7 ± 0.3 2.7 ± 0.3 1.000</td>
</tr>
<tr>
<td>5</td>
<td>1.7 ± 0.9 2.3 ± 0.7 0.184</td>
<td>2.0 ± 0.0 2.3 ± 0.3 0.423</td>
</tr>
<tr>
<td>6</td>
<td>0.0 ± 0.0 0.0 ± 0.0 1.000</td>
<td>0.7 ± 0.3 1.0 ± 0.6 0.423</td>
</tr>
<tr>
<td>7</td>
<td>0.0 ± 0.0 0.0 ± 0.0 1.000</td>
<td>0.7 ± 0.3 1.0 ± 0.6 0.423</td>
</tr>
<tr>
<td>8</td>
<td>0.3 ± 0.3 0.3 ± 0.3 1.000</td>
<td>0.0 ± 0.0 0.3 ± 0.3 0.423</td>
</tr>
<tr>
<td>9</td>
<td>0.3 ± 0.3 0.3 ± 0.3 1.000</td>
<td>0.3 ± 0.3 0.3 ± 0.3 1.000</td>
</tr>
<tr>
<td>10</td>
<td>0.0 ± 0.0 0.0 ± 0.0 1.000</td>
<td>0.7 ± 0.3 1.0 ± 0.6 0.423</td>
</tr>
<tr>
<td>11</td>
<td>0.7 ± 0.3 0.7 ± 0.3 1.000</td>
<td>0.0 ± 0.0 0.0 ± 0.0 1.000</td>
</tr>
<tr>
<td>12</td>
<td>0.0 ± 0.0 0.3 ± 0.3 0.423</td>
<td>1.0 ± 0.0 1.0 ± 0.0 1.000</td>
</tr>
<tr>
<td>13</td>
<td>0.0 ± 0.0 0.3 ± 0.3 0.423</td>
<td>0.0 ± 0.0 0.0 ± 0.0 1.000</td>
</tr>
<tr>
<td>14</td>
<td>0.0 ± 0.0 0.0 ± 0.0 1.000</td>
<td>0.7 ± 0.3 0.7 ± 0.3 1.000</td>
</tr>
<tr>
<td>16</td>
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<td>0.0 ± 0.0 0.0 ± 0.0 1.000</td>
</tr>
<tr>
<td>18</td>
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<td>0.3 ± 0.3 0.7 ± 0.3 0.423</td>
</tr>
<tr>
<td>21</td>
<td>0.7 ± 0.3 0.7 ± 0.3 1.000</td>
<td>0.3 ± 0.3 0.7 ± 0.7 0.423</td>
</tr>
<tr>
<td>28</td>
<td>0.0 ± 0.0 0.0 ± 0.0 1.000</td>
<td></td>
</tr>
<tr>
<td>32</td>
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</tr>
<tr>
<td>35</td>
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<td></td>
</tr>
<tr>
<td>38</td>
<td>0.3 ± 0.3 0.3 ± 0.3 1.000</td>
<td></td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>44</td>
<td>0.0 ± 0.0 0.0 ± 0.0 1.000</td>
<td></td>
</tr>
<tr>
<td>46</td>
<td>0.0 ± 0.0 0.7 ± 0.3 0.184</td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>0.3 ± 0.3 0.3 ± 0.3 1.000</td>
<td></td>
</tr>
</tbody>
</table>

Similarity indices, however, revealed that the communities collected in the qualitative sample often did not include rarer, less numerous taxa. For example, on day 3 of succession in the first trial, the qualitative samples from all three replicates included only the ant *Myrmica* sp. or *Sarcophaga* sp., whereas calliphorid eggs and sarcophagid first- and second-stage larvae were also recovered from natural orifices (mouth and anus) of the DS carcasses during processing (although these areas had been searched during the qualitative sampling in the field). Other examples included taxa that were present in very small numbers throughout the succession (*L. alienus*, *Saprinus* sp., *T. lapponicus*). Later in the succession, both species of Dermestes were frequently present, but only one or the other was collected in the qualitative samples. On 10 visits, there were no insects present on the carcasses (days 0 and 1, then sporadically after day 6).
These data have implications for the estimation of the postmortem interval in human forensic casework. Schoenly et al. (1996) demonstrated that postmortem interval estimates based on carrion-arthropod succession data are sensitive to the number of taxa in the data set, where fewer taxa increased the width of the confidence interval of the postmortem interval. Although the number of taxa collected in this study did not show significant differences between qualitative samples and the actual number of taxa present, the communities collected were frequently of different taxa and postmortem interval estimates resulting from differing taxa sets might vary as a result.

**Effects of disturbance**

There were no statistically significant differences between number of taxa in the qualitative samples from the DS carcasses and from the P carcasses ($P \geq 0.184$) or between the number of taxa in qualitative subsamples from the DS carcasses and from the P & W carcasses ($P \geq 0.130$) for either trial (Tables 3 and 4).

**Table 3.** Number of taxa (mean ± SE) in qualitative subsamples from destructively sampled (DS), fauna only sampled (P) and fauna and biomass sampled (P & W) carcasses exposed 22 June 2002. *P*-values are from unpaired *t*-tests comparing data from the corresponding category vs. DS carcasses, *n* = 3.

<table>
<thead>
<tr>
<th>Day</th>
<th>DS carcasses</th>
<th>P carcasses</th>
<th><em>P</em>-value</th>
<th>P &amp; W carcasses</th>
<th><em>P</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>1.000</td>
<td>0.0 ± 0.0</td>
<td>1.000</td>
</tr>
<tr>
<td>1</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>1.000</td>
<td>0.0 ± 0.0</td>
<td>1.000</td>
</tr>
<tr>
<td>2</td>
<td>1.7 ± 0.3</td>
<td>1.7 ± 0.3</td>
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<td>0.653</td>
</tr>
<tr>
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<td>1.7 ± 0.7</td>
<td>0.635</td>
</tr>
<tr>
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<td>0.225</td>
<td>0.7 ± 0.3</td>
<td>0.423</td>
</tr>
<tr>
<td>6</td>
<td>0.0 ± 0.0</td>
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<td>0.225</td>
<td>0.3 ± 0.3</td>
<td>0.423</td>
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<tr>
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<td>1.000</td>
<td>0.3 ± 0.3</td>
<td>0.423</td>
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<tr>
<td>8</td>
<td>0.3 ± 0.3</td>
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<td>0.423</td>
<td>0.3 ± 0.3</td>
<td>1.000</td>
</tr>
<tr>
<td>9</td>
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<td>0.423</td>
<td>0.0 ± 0.0</td>
<td>0.423</td>
</tr>
<tr>
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<td>0.423</td>
<td>0.0 ± 0.0</td>
<td>1.000</td>
</tr>
<tr>
<td>11</td>
<td>0.7 ± 0.3</td>
<td>0.0 ± 0.0</td>
<td>0.184</td>
<td>0.3 ± 0.3</td>
<td>0.667</td>
</tr>
<tr>
<td>12</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>1.000</td>
<td>0.3 ± 0.3</td>
<td>0.423</td>
</tr>
<tr>
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<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>1.000</td>
<td>0.0 ± 0.0</td>
<td>1.000</td>
</tr>
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<td>0.0 ± 0.0</td>
<td>1.000</td>
<td>0.7 ± 0.7</td>
<td>0.423</td>
</tr>
<tr>
<td>16</td>
<td>0.3 ± 0.3</td>
<td>0.0 ± 0.0</td>
<td>0.423</td>
<td>0.3 ± 0.3</td>
<td>1.000</td>
</tr>
<tr>
<td>18</td>
<td>0.7 ± 0.3</td>
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<td>2.3 ± 0.3</td>
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<tr>
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<td>1.000</td>
</tr>
<tr>
<td>32</td>
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<td>0.0 ± 0.0</td>
<td>1.000</td>
</tr>
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<td>35</td>
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<td>0.0 ± 0.0</td>
<td>1.000</td>
<td>0.0 ± 0.0</td>
<td>1.000</td>
</tr>
<tr>
<td>38</td>
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<td>0.423</td>
<td>0.0 ± 0.0</td>
<td>1.000</td>
</tr>
<tr>
<td>41</td>
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<td>1.000</td>
<td>0.0 ± 0.0</td>
<td>1.000</td>
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<tr>
<td>44</td>
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<td>0.0 ± 0.0</td>
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<td>0.0 ± 0.0</td>
<td>1.000</td>
</tr>
<tr>
<td>46</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>1.000</td>
<td>0.7 ± 0.3</td>
<td>0.423</td>
</tr>
<tr>
<td>49</td>
<td>0.3 ± 0.3</td>
<td>0.7 ± 0.3</td>
<td>0.423</td>
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</tr>
</tbody>
</table>
Table 4. Number of taxa (mean ± SE) in qualitative subsamples from destructively sampled (DS), fauna only sampled (P), and fauna and biomass sampled (P & W) carcasses exposed 20 July 2002. 

<table>
<thead>
<tr>
<th>Day</th>
<th>DS carcasses</th>
<th>P carcasses</th>
<th>P-value</th>
<th>P &amp; W carcasses</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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<td>0.0 ± 0.0</td>
<td>1.000</td>
<td>0.0 ± 0.0</td>
<td>1.000</td>
</tr>
<tr>
<td>1</td>
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<td>0.7 ± 0.3</td>
<td>0.423</td>
<td>1.0 ± 0.0</td>
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</tr>
<tr>
<td>2</td>
<td>2.0 ± 0.0</td>
<td>2.0 ± 0.6</td>
<td>1.000</td>
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<td>0.423</td>
</tr>
<tr>
<td>3</td>
<td>1.7 ± 0.7</td>
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<tr>
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<td>1.000</td>
<td>1.7 ± 0.3</td>
<td>0.423</td>
</tr>
<tr>
<td>6</td>
<td>0.7 ± 0.3</td>
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<td>0.7 ± 0.3</td>
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<td>7</td>
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</tr>
<tr>
<td>8</td>
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<td>1.000</td>
<td>0.3 ± 0.3</td>
<td>0.423</td>
</tr>
<tr>
<td>9</td>
<td>0.3 ± 0.3</td>
<td>0.0 ± 0.0</td>
<td>0.423</td>
<td>0.0 ± 0.0</td>
<td>0.423</td>
</tr>
<tr>
<td>10</td>
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<td>1.3 ± 0.3</td>
<td>0.184</td>
<td>1.0 ± 0.6</td>
<td>0.423</td>
</tr>
<tr>
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<td>0.3 ± 0.3</td>
<td>0.423</td>
<td>0.3 ± 0.3</td>
<td>0.423</td>
</tr>
<tr>
<td>12</td>
<td>1.0 ± 0.0</td>
<td>1.3 ± 0.3</td>
<td>0.423</td>
<td>0.7 ± 0.3</td>
<td>0.423</td>
</tr>
<tr>
<td>13</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>1.000</td>
<td>0.3 ± 0.3</td>
<td>0.423</td>
</tr>
<tr>
<td>14</td>
<td>0.7 ± 0.3</td>
<td>1.0 ± 0.0</td>
<td>0.423</td>
<td>0.7 ± 0.3</td>
<td>1.000</td>
</tr>
<tr>
<td>16</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>1.000</td>
<td>0.3 ± 0.3</td>
<td>0.423</td>
</tr>
<tr>
<td>18</td>
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<td>21</td>
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<td>0.0 ± 0.0</td>
<td>0.423</td>
<td>0.3 ± 0.3</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Again, however, the community similarity index showed considerable differences in community composition between treatments. From day 6 of succession to the end of the study, collections with zero similarity were not uncommon. Those visits had low community similarities primarily because the taxa collected were generally present in densities of only one or two specimens per carcass (esp. *Dermestes* spp.). VanLaerhoven & Anderson (1999) found a similar lack of statistically significant differences between three buried pig carcasses disinterred and reburied at 2 weeks and three other buried pig carcasses when both were exhumed 6 weeks after initial burial. They reported minor differences in community composition but were unable to draw conclusions as to overall effects of repeated disturbance due to the limited sample size. Their study also included a much larger number of taxa.

Conclusions

The results from this series of experiments indicate that investigator disturbance does not have a significant overall impact on the number of taxa sampled from rat carcasses in forensic entomology experiments. A total of 39 invertebrate taxa were collected over the course of the investigation, a total intermediate to that seen in other studies from the arid/semiarid southwestern United States. Early stages of decomposition were dominated by the Sarcophagidae, both in terms of density (number of organisms) and biomass. Two taxa of
Dermestidae were collected throughout succession, whereas other invertebrate taxa appeared more sporadically. Community composition on the carcasses was more similar between treatments in visits early in the decomposition process, with more disparate communities in later visits due to small numbers of rare taxa.

Although the results found in the present study appear to validate methods used historically and in the present, care should still be taken to determine that experimental data are collected appropriately. For example, every orifice of a carcass should be diligently searched for invertebrates, sampling should have a minimal effect on the total community, carcasses should be manipulated as little as possible to avoid excess disturbance of the community, etc. Further research investigating the validity of current experimental techniques could involve larger carcasses (particularly pigs for standardization, as in other experimental work, or comparability to humans), or sampled more intensively during the active decomposition stages to determine if the same patterns exist.

This study was conducted with small mammal carcasses (< 210 g) in a hot, dry climate on the Great Plains of Colorado. Decomposition of rodent carcasses has been vastly different when carcasses are exposed to environments such as temperate forests (Bornemissza, 1957; Nabaglo, 1973; Johnson, 1975; Kentner & Streit, 1990; Isiche et al., 1992; Kocárek, 2003). Although many of the taxa associated with these small carcasses are the same as are encountered with larger carriions, including human corpses, the pattern of succession of these organisms on rat carcasses does not necessarily reflect that which may be encountered in forensic entomology casework (Hewadikaram & Goff, 1991). Indeed, this study was undertaken primarily as an examination of procedures commonly used in experimental forensic entomology (using replication with small carcasses more likely to show statistically significant results) and not to provide extensive succession data for medico-legal use.

Acknowledgments – We would like to 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, Steve Canton and Don Conklin, Jr. provided resources for use in the field and for analysis of data. Boris Kondratieff, Paula Cushing, and Bruce Cutler provided some specimen identifications or confirmations. Jim Chadwick and John Foster provided advice on the project in its initial and final stages, respectively. The work described herein was conducted in partial fulfillment of the requirements for the degree of Master of Science by G.D.D. through the University of Nebraska–Lincoln, advised by W. Wyatt Hoback, Leon Higley, and John Foster. This is Journal #15073 of the University of Nebraska.

References


