Carcass Enrichment Does Not Alter Decay Rates or Arthropod Community Structure: A Test of the Arthropod Saturation Hypothesis at the Anthropology Research Facility in Knoxville, Tennessee

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J. Med. Entomol. 40(4): 559–569 (2003)

ABSTRACT In a test of an arthropod saturation hypothesis, we asked if the 30-yr history of carcass enrichment at the Anthropology Research Facility, Knoxville TN, has altered carcass decay rates or community structure of sarcosaprophagous arthropods, compared with three local nonenriched sites. Over a 12-d period in 1998, using pitfall traps and sweep nets, we sampled a total of 81,000 invertebrates from freshly euthanized pigs (*Sus scrofa* L.) placed in these sites. From this number, we sorted 69,286 forensically important (sarcosaprophagous) arthropods. The community structure of these organisms, as measured by species and individuals accumulation curves, rarefaction, and nonparametric correlation, was comparable in all four sites in taxonomic similarity, colonization rates, aerial species richness, and ranked abundances of forensically important taxa on a per carcass basis. Measures of carcass decay rate, remaining carcass weight (%) and periodic weight loss, also were similar. In most cases, carcass surface temperatures and maggot mass temperatures were also statistically indistinguishable. Probability-based results and posthoc power analyses of these variables led us to conclude that the sarcosaprophagous arthropod community of the Anthropology Research Facility is representative of surrounding sites.

KEY WORDS nutrient enrichment, Anthropology Research Facility, carrion-arthropod succession, forensic entomology

NUTRIENT ENRICHMENT, the input of nutrients by natural or anthropogenic means, impacts terrestrial and aquatic ecosystems by impoverishing biological communities (e.g., Molles 1999). Like polluted ecosystems, nutrient-enriched ecosystems can become dominated by the most tolerant or competitive species, thus altering both species richness and evenness of the biological community (Kempton 1979, Molles 1999). Studies of whole-lake responses to eutrophication (Cole 1994, Wetzel 2001), plant diversity losses in fertilized plots (Leigh and Johnston 1994), and correlational evidence that increasing nutrient availability reduces plant, algae, and diatom richness (Molles 1999) demonstrate that primary producers (and other trophic groups) are impacted in similar ways to different types of nutrient enrichment. Despite heightened interest in ecological disturbances and their causes, consequences, and benefits (Freedman 1995, Liddle 1997, Reice 2001), limited attention has been paid to understanding impacts of nutrient enrichment on terrestrial arthropod communities; examples include terrestrial oil spills (Freedman 1995), dung accumulation (Williams et al. 1985, Hanski and Cambefort 1991), fallen trees (Maser et al. 1984), and catastrophic animal mortality (Weigelt 1989, Lyman 1994). In the aftermath of natural or anthropogenic disasters, human remains (when left undiscovered) can suffer the same postmortem fate as those of other animals; beyond their entomological and ecological context, such events have implications to public health (Stonier 1964, Moeller 1997, Maxcy et al. 1998), forensic taphonomy (Haglund and Sorg 1997, 2002), paleoanthropology (Wolpoff 1998), and bioterrorism (Knobler et al. 2002, Wheelis et al. 2002).

Carrion-arthropod systems are useful and informative models for studying impacts of nutrient enrichment because much is already known of their biology through entomological (Hall 1948, Greenberg 1971, 1973), ecological (Putnam 1983), and forensic (Smith

The protocol for the euthanasia of pigs used as carrion in this research was approved by the Animal Care and Use Committee, University of Missouri. Investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

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0022-2585/03/0559–0569$04.00/0

1986, Catts and Haskell 1990, Byrd and Castner 2001) sources. As discrete and ephemeral resources, vertebrate carcasses are nutrient-rich substrates for microbial, invertebrate, and vertebrate populations that are amenable to replication and sampling (Schoenly and Reid 1987). Invertebrates alone can exceed 500 taxa in carcasses (Payne and Crossley 1966). Individually and collectively, they drive the pattern, process, and rate of soft-tissue decomposition, endogenous heat production, heterotrophic succession, liquefaction, and (partial) skeletal disarticulation of the remains.

The Anthropology Research Facility (ARF) at the University of Tennessee, Knoxville, is devoted to the scientific study of postmortem human decay. Throughout its 30-yr history, more than 400 sets of human remains have been studied at ARF, including insect faunal surveys (Rodriguez and Bass 1983, 1985), and comparative field experiments with dead pigs as model corpses (Schoenly et al. 1996, Haskell et al. 2001). Yet it remains unclear whether ARF, with its history of carcass enrichment, is comparable to nonenriched sites in carcass decay rates and arthropod community structure. One might predict that such enrichment would saturate this location with sarcosaprophagous arthropods of reduced diversity, and that this would modify the rate of cadaver decomposition.

The absence of any experimental tests of this "arthropod saturation hypothesis" led us to conduct a comparative field test at ARF and at three sites various distances away. Our approach used pigs as model corpses, conventional statistics to test between-site differences in carcass decay rate, carcass surface temperature, and maggot mass temperature, and measures of community structure applied to invertebrate time-series data from pitfall traps and sweep nets. In particular we sought evidence of (1) faster carcass decay rates, (2) impoverished community structure with fewer but larger populations of sarcosaprophagous arthropods, and (3) faster colonization rates of arthropod species and individuals, relative to surrounding sites.

Materials and Methods

Field Sites. The study was conducted in the summer of 1998 at four sites designated S1 through S4, in and around the Knoxville area of southeastern Tennessee. S1, the Anthropology Research Facility, is a fenced 1.6-ha wooded area <200 m from the Tennessee River. Fieldwork was conducted on the northwest-facing slope of the facility. S1 personnel deposited six cadavers in the site during spring 1998 that were skeletonized or mummified at the time of our 12-d study. Remains of older cadavers were also present.

S2 is a wooded hill separated from a cow pasture by a fence and is ≈700 m southwest of S1. Fieldwork was conducted on a west-facing slope, 35 m from a large pond.

S3 is a wooded hill on a slight west-facing slope within the University of Tennessee Agriculture Experiment Station, Plant Science Unit, ≈6 km southeast of S1.

S4 is farmland located in Norris, TN, and ≈40 km north of S1. Fieldwork was conducted on a N-facing rocky hill surrounded by coniferous and deciduous trees.

Pig Placement. Three freshly euthanized pigs (*Sus scrofa* L.) were distributed 1.8 to 2.5 m apart in a linear arrangement at each site. Pigs were euthanized by intracardial injection of sodium pentobarbital (Beuthanasia-D, Schering-Plough Animal Health Corporation, Union, NJ). Carcass placement occurred before sunrise to synchronize arthropod visitation times across sites (Nuorteva 1977). Starting weights (kg) and gender of pigs were randomized across sites (mean ± SD): S1 (23.7 ± 0.58; 3 males), S2 (26.0 ± 1.0; 2 males, 1 female), S3 (25.3 ± 2.31; 2 males, 1 female), and S4 (26.7 ± 1.53; 2 males, 1 female).

Arthropod Sampling. Ground and flying arthropods were sampled from pigs using pitfall traps and sweep nets from 19 June to 30 June 1998. Daily hand samples were collected at arbitrary times each day by one or more persons but were only used to record the presence or absence of a species on a carcass. Studies of arthropod succession at S1 indicate that 88% of the forensically important taxa and 81% of their individuals occur on pig remains within 12 d of placement (N.H.H., K.S., R.D.H., unpublished data).

Pitfall trap and sweep net methods conformed to standard protocols (Catts and Haskell 1990). Four pitfall traps were buried around a pig at S1–S3 along the four cardinal directions (pig B); the rocky hillside at S4 permitted only one pitfall trap to be installed near the three carcasses. A fifth pitfall trap was spaced 75–100 m away from the nearest pig at S1–S3, functioning as a background control. Pitfall traps were constructed from a 20-cm section of 10-cm diameter PVC pipe, a 1-pint wide-mouth canning jar, and a 10-cm maximum diameter plastic funnel with the stem removed. The PVC pipe was buried vertically with its top edge at ground level. Each jar was filled 4 cm deep with 95% ethanol, placed into a PVC pipe, then covered with the funnel. For aerial arthropods, three repetitive sweeps of a sweep net were made in rapid succession over the carcass. Sweep samples were separated by 5-min intervals to allow arthropod resettlement on the carcass. Collected materials from both methods were flushed into coded vials containing 75% ethanol.

Environmental Variables. Habitat and carcass temperatures were sampled daily at each site using a digital pocket thermometer (TempTestr, Seedburo Equipment Company, Chicago, IL). Temperature data included ambient air temperature, carcass surface temperature, and, where possible, maggot mass temperature. A 4.5-h traveling and sampling window prevented all four sites from being sampled at the same time of day. To reduce interaction effects between site order and temperature variables, sites were assigned randomized sampling times of 1300, 1430, 1600, or 1730 hours. Because each day of the study was analyzed separately for temperature differences among sites,

this 4.5-h sampling window did not materially alter our results.

To facilitate daily weighing, two pigs at each site were placed on separate 10 × 8-cm mesh wire screens. Each pig was weighed using a free-action weighing device. Changes in mean weight of the two pigs in each site (kg) were calculated two different ways: (1) by dividing mean current weight over mean starting weight, then multiplied by 100 to obtain the percentage of remaining carcass weight, and (2) by subtracting mean weights between consecutive sampling dates, then dividing by mean starting weight to obtain periodic weight loss.

Invertebrate Identifications. Using a stereo dissecting microscope, organisms were sorted to stage (immatures, adults) and identified to the lowest possible taxon, usually genus and species for adults of forensically important taxa. Arachnids, chilopods, and diplopods were identified to order; all other (noninsect) invertebrates were identified to class. We used the following keys: Hall and Townsend (1977), McAlpine (1981), Stehr (1987), and Borror et al. (1992). All identified specimens were sealed into labeled patentlip vials containing 75% ethanol. Stratiomyid (*Ptecticus* spp.) eggs were accidentally undercounted and thrown away before final spreadsheet entry. Voucher specimens were deposited in the W.R. Enns Entomology Museum at the University of Missouri.

Statistical Analysis. Because pitfall traps within sites were replicated at only three of the four sites, daily counts were averaged for S1–S3 and rounded to the nearest integer; daily counts from the single pitfall trap at S4 were left unchanged. Site means for sweep-net catches (three sweeps per site) were similarly calculated. Pairwise differences between S1 and the other three sites were assessed using a combination of graphical methods, conventional statistics, and community-level ecostatistics:

(a) Repeated measures on daily carcass temperatures (surface and maggot mass) and periodic weight loss were analyzed using split-plot analysis of variance (ANOVA) (Gill and Hafs 1971, von Ende 2001), which treats days as a subunit time effect. This approach recognizes that there is variance and covariance of correlated data resulting from repeated measures of the same variable (carcass) at the same sites. This approach is valid only if uniformity tests (Saavedra and Douglass 2002) confirm that measures taken closer in time (e.g., days 1 versus 2), are not more correlated than those taken farther apart in time (e.g., days 1 versus 12). Consequently, we first employed a type III ANOVA on the raw or transformed environmental variables that treated sites as the fixed factor and carcasses and days as random factors. If the interaction between sites and days was significant, we resorted to least significant difference (LSD) tests on each day to determine which site(s) differed.

(b) Comparison of arthropod abundances in pitfall traps and sweep nets was achieved using matched rank-abundance plots (Longino and Colwell 1997) in which one method (i.e., pitfall catches) was chosen as the reference plot to which the other method (sweep net) is compared. When plotted in this way, rank-abundance plots permit a quick visual check of the degree of correspondence between two sampling methods (Longino and Colwell 1997).

(c) Species accumulation (or yield-effort) curves show the rate of colonization of species or individuals over time (Schoenly et al. 1992). Accumulation curves were plotted separately for pitfall and sweep-net catches using forensically important taxa, averaged over the replicates at each site. These curves plot the sums of the number of taxa (or individuals of taxa) in the previous sample and the number of taxa (or number of individuals of taxa) in the present sample that were not observed in any previous sample. For the first sample, the cumulative numbers of taxa (or cumulative number of individuals) are defined to equal its numbers of taxa (or its numbers of individuals).

(d) $E_{S(n)}$, the rarefaction diversity statistic and its variance [$var\ E_{S(n)}$] were used to test the null hypothesis that two or more samples have been drawn from the same parent population (Hurlbert 1971, Simberloff 1972). Rarefaction assumes a random spatial dispersion of individuals (Simberloff 1978). To validate this model for carrion-invertebrate data, we compared rarefaction curves of pitfall and sweep-net catches against randomized collector's curves (Colwell and Coddington 1994). Variation in curve shape for collector's curves arises from heterogeneity among the units sampled (and from sampling error; see Colwell and Coddington 1994). To eliminate this problem, sample order was randomized 1000 times and the mean and standard deviation of $E_{S(n)}$ were computed for each value of n along the x-axis. Visual inspection of both curves for each sampling method show that every point of the rarefaction curves fell neatly within the 95% confidence bands of the randomized collector's curves (Fig. 1A and B). Thus, the sampling-without replacement version of the rarefaction model (Simberloff 1972) is a reasonable sampling model for representing sweep-net and pitfall-trap catches in carrion. Between-site analysis relied on complete rarefaction curves bracketed by bands 1.96 standard deviations wide (i.e., approximately equal to its 95% CL) for pitfall and sweep-net catches using the sites with the largest total abundance. The null hypothesis was upheld if data points for all three remaining sites fell within the 95% confidence bands of the rarefaction curve.

(e) *Rs*, Spearman (1904) rank test, and its correction for excessive ties (Daniel 1990), was used to test the null hypothesis that species-abundance rankings from site pairs were independent (Daniel 1990). Rank tests were calculated separately for pitfall and sweep-net samples and for every pairwise combination of sites that included S1.

Because (a) and (e) above each addresses a common null hypothesis, we considered dates and sites as part of one 12-d experiment. Consequently, to minimize type 1 errors that might arise as a result of the high number of pairwise comparisons, alphas ($P < 0.05$) for all correlations were adjusted using the Bonferroni multiple-test procedure (Miller 1981, Rice

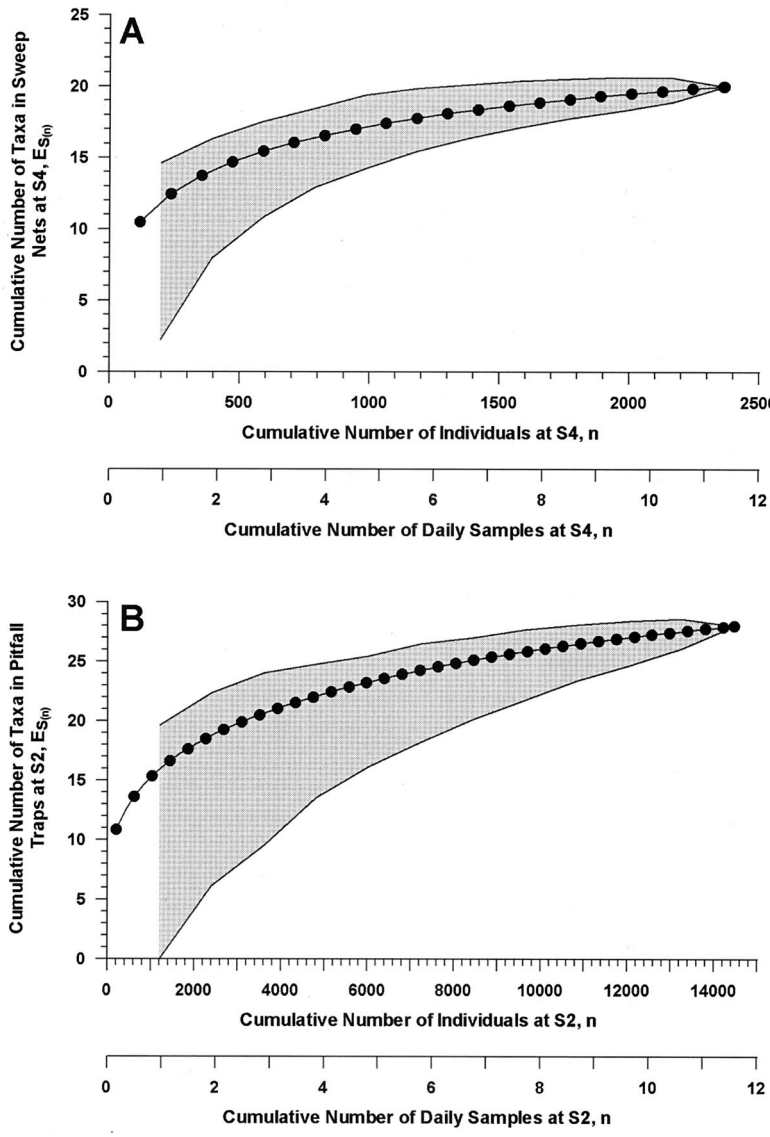

Fig. 1. Validation of rarefaction (beaded line) as a sampling model for forensically-important taxa, verified by collector's curves (represented by its 95% CL), taken from sweep nets (A) and pitfall traps (B) using sites with the highest total abundance. Confidence limits for collectors' curves are the result of 1000 randomizations of sample pooling order.

1989), such that if a collection of k tests is simultaneously carried out, the Bonferroni adjustment is equal to α/k.

Power is the probability of rejecting the null hypothesis (i.e., carcass enrichment does not affect decomposition parameters or arthropod community structure) when it is false (Zar 1984). The statistical power of a given test depends on the sample size (n), the amount of between-replicate variability (σ), and the magnitude of the measured difference between treatments. Of these, sample size is the only variable an experimenter can most directly control. In this study, a posthoc power analysis was conducted on three variables (carcass weight loss, pitfall-trap catches, sweep-net catches) to determine what sample size would be needed in a future study to detect significant (between-site) differences of the magnitude observed in our study with $\alpha = 0.05$ and $\beta = 0.10$. Estimation of power was performed using PASS 6.0 software (J.L. Hintze, 329 North 1000 East, Kaysville, UT 84037).

Results

Environmental Variables. Carcasses did not begin to lose weight until the third day (Fig. 2) and maggot masses were only observable on days 3–8. Interaction of days and sites was highly significant for carcass surface temperature (type III ANOVA; $F = 3.94$, df = 3, $P = 0.0001$), maggot mass temperature ($F = 2.17$, $P =$

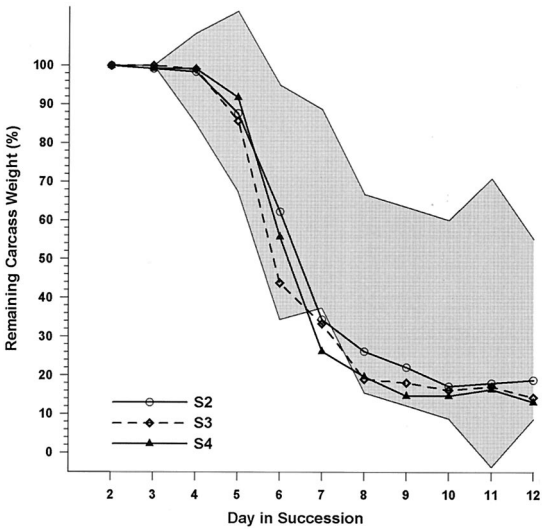

Fig. 2. Percentage of remaining carcass weight as a function of time at each site. The curve for S1 (shaded region) is the mean bracketed by a band 1.96 standard deviations wide, which constitutes approximate 95% confidence intervals. Each curve is based on means, over two carcasses, for each site.

0.0315) and periodic weight loss ($F = 5.51, P = 0.0001$). Thus, sites manifested substantial variation in carcass microclimate over the 12 days of this study. On an experiment-wide level, LSD tests showed that S2–S4 were statistically comparable to *S1* for all three environmental variables in a large majority of cases: carcass surface temperatures (29 of 36 tests [81%], Table 1), maggot mass temperatures (15 of 17 tests [88%], Table 2), and periodic weight loss (25 of 30 [83%], Table 3). Site by site analysis of these three variables revealed that S2 was the most comparable to S1 (89% of 28 tests), followed closely by S4 (86%), and then S3 (79%) (Tables 1–3).

Table 1. *P*-values of least significant difference (LSD) tests of mean carcass surface temperatures, taken over three carcasses, for each pairwise combination of sites that included S1

Date (day in study)	Site comparison		
	S1 vs. S2	S1 vs. S3	S1 vs. S4
19 June (1)	0.2976	0.1196	**0.0002**
20 June (2)	0.6016	**0.0001**	0.1937
21 June (3)	0.1196	0.0050	0.7940
22 June (4)	0.2976	**0.0004**	0.0701
23 June (5)	0.0701	1.0000	0.0701
24 June (6)	0.1937	0.0104	**0.0004**
25 June (7)	0.2976	0.0701	0.0206
26 June (8)	0.1196	**0.0004**	0.0206
27 June (9)	1.0000	0.0701	1.0000
28 June (10)	0.7940	0.0050	0.2976
29 June (11)	0.2976	**0.0001**	0.4340
30 June (12)	0.0701	**0.0001**	0.1196

Note: Numbers in bold type are significant at the 0.05 level using Bonferroni adjustment for multiple test procedures. Because these results share a common null hypothesis and dates and sites significantly interact (see Results: Environmental Variables), these variables were considered dependent.

Table 2. *P*-values of least significant difference (LSD) tests of mean maggot mass temperatures, taken over three carcasses, on days 3–8 for each pairwise combination of sites that included S1. Maggot masses were only observable on days 3–8 of this study

Date (day of study)	Site comparison		
	S1 vs. S2	S1 vs. S3	S1 vs. S4
21 June (3)	0.7304	0.1270	0.3911
22 June (4)	0.4918	0.3911	0.4918
23 June (5)	0.6056	0.6056	0.7304
24 June (6)	1.0000	0.7304	0.4918
25 June (7)	**0.0001**	0.0203	**0.0022**
26 June (8)	0.0162	no mass	0.0191

Note: Numbers in bold type are significant at the 0.05 level using Bonferroni adjustment for multiple test procedures. Because these results share a common null hypothesis and dates and sites significantly interact (see Results: Environmental Variables), these variables were considered dependent.

Plots of remaining carcass weight, expressed as a percentage of mean starting weight (Fig. 2), corroborated statistical (LSD) results of periodic weight loss (Table 3). Variation in remaining carcass weights increased at S1 after day 7, thus widening its 95% confidence bands (shaded region, Fig. 2). Compared with the other sites, carcasses at S1 decomposed at a slower rate, starting on day 6 and ending on day 10. Afterward, carcasses regained some weight on days 11–12 as a result of rehydration from precipitation (Fig. 2). Carcass weights at S2–S4 fell inside S1's 95% confidence bands on 27 of 33 site-days (82%).

If the variance estimates of remaining carcass weight from the pigs are representative of what the next study will find, that study will require a high number of replicates to have sufficient power (i.e., 90%) in detecting small differences between S1 and the other sites. For example, a difference of 15% weight loss in the pig carcasses observed between S1 and the other sites on day 12 would require no fewer than seven carcasses per site (instead of the two carcasses measured in the current study) to give a 90%

Table 3. *P*-values of least significant differences (LSD) tests of mean periodic weight loss of carcasses, taken over carcasses A and C on days 3–12 for each pairwise combination of sites that included S1. Carcasses did not show weight loss until day 3

Date (day of study)	Site comparison		
	S1 vs. S2	S1 vs. S3	S1 vs. S4
21 June (3)	0.8281	1.0000	0.8176
22 June (4)	0.4818	0.5073	0.3519
23 June (5)	0.2070	0.0520	0.6842
24 June (6)	0.8387	**0.0001**	0.0096
25 June (7)	**0.0001**	0.0188	**0.0001**
26 June (8)	**0.0007**	0.0449	**0.0002**
27 June (9)	0.8387	0.5247	0.6546
28 June (10)	0.6743	0.7042	0.3659
29 June (11)	0.8176	0.7757	0.6449
30 June (12)	0.5159	0.7244	0.6546

Note: Numbers in bold type are significant at the 0.05 level using Bonferroni adjustment for multiple test procedures. Because these results share a common null hypothesis and dates and sites significantly interact (see Results: Environmental Variables), these variables were considered dependent.

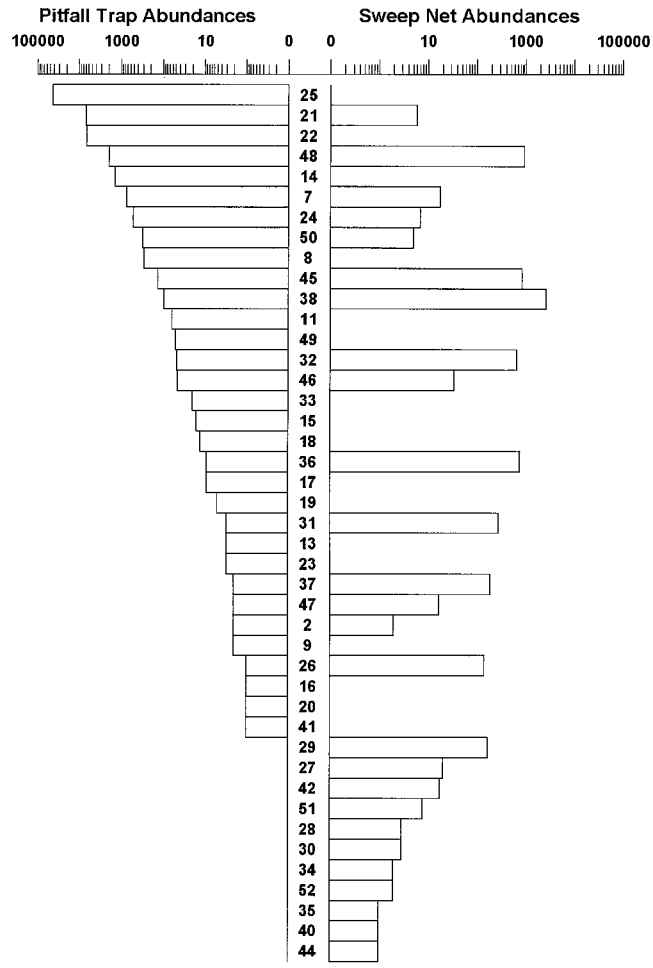

Fig. 3. Matched rank-abundance plots of forensically important taxa collected from sweep-net and pitfall-trap samples, totaled over all sampling dates. The left side is a standard rank-abundance plot for pitfall samples drawn vertically with the most abundant taxa at the top; the right side shows the corresponding abundances for sweep-net samples. The identity of each taxon, listed by its identification number (2–52 noninclusive), is given in the Appendix.

likelihood of rejecting the null hypothesis at an alpha level of 0.05.

Rank-Abundance Plots. In excess of 81,000 invertebrates were collected and identified over the 12-d period, representing 26 orders, 118 families, and 223 taxa. Based on lists provided in Catts and Haskell (1990) and Schoenly (1992), we then separated each known forensically useful taxon, then totaled arthropod abundances over all sampling dates. This procedure reduced the pitfall and sweep-net counts to 62,460 and 6,826 individuals, respectively, for a total sarcosaprophagous fauna of 69,286 individuals.

Pitfall traps and sweep nets were nearly equally efficient in capturing high percentages of forensically important organisms (84% versus 93%, respectively) even though pitfall traps caught 10 times more individuals than sweep nets over the 12-d period (Fig. 3). Matched rank-abundance plots for pitfall and sweep-net catches show a high degree of overlap in forensically important taxa and show that some taxa that were common in one method were common in the other. Conversely, matched abundance plots show that a few species, caught as singletons by one method (e.g., taxa represented as single specimens), were caught either in moderately large numbers or not at all by the other method (Fig. 3).

Species Accumulation Rates. Cumulative numbers of forensically important taxa and individuals, calculated as percentages to allow between-site comparisons, were plotted separately for sweep-net and pitfall-trap samples (Fig. 4A–D). Ninety-five percent of the sweep-net taxa were captured at S1 by day 9, whereas 95% of the sampled individuals were caught by day 4. In pitfall traps, 95% of the sampled taxa at S1 were captured within 11 d whereas 95% of individuals were caught in 8 d. In both methods, the steeper rise for individuals (Fig. 4B and D) as compared with taxa (Fig. 4A and C) coincided with the arrival of large

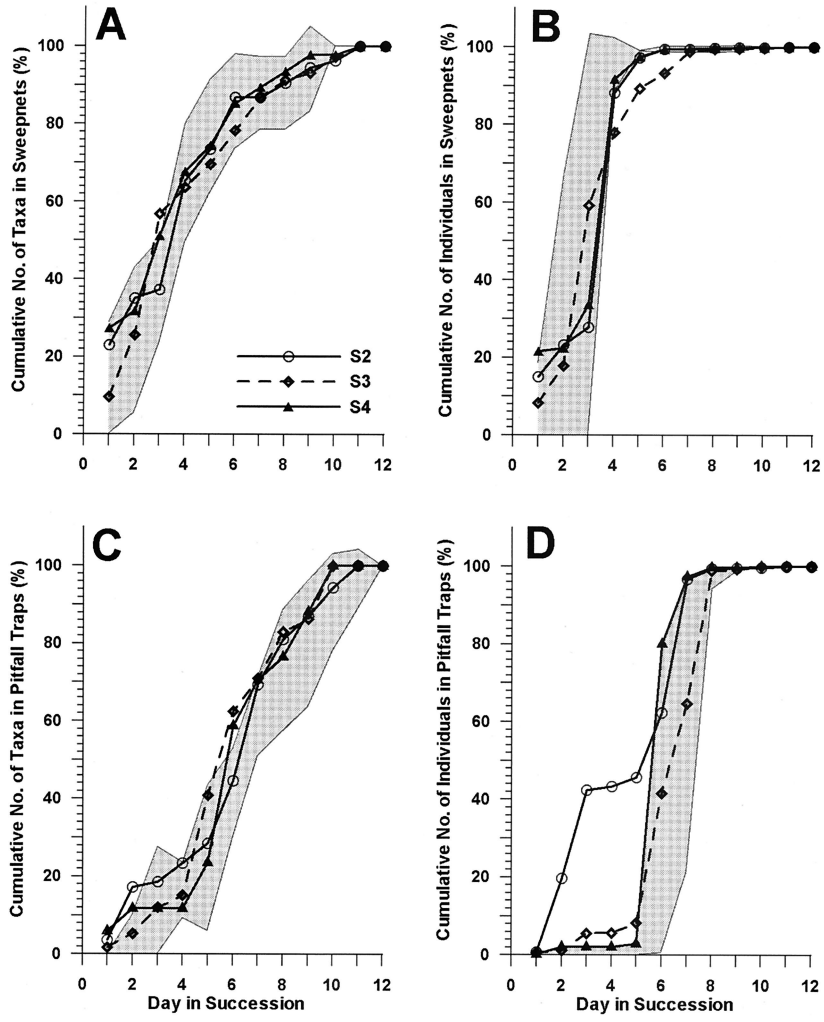

Fig. 4. Cumulative number of taxa and individuals for sweep-net (A-B) and pitfall trap (C-D) samples. The curve for S1 (shaded region) is the mean bracketed by a band 1.96 standard deviations wide, which constitutes approximate 95% confidence intervals. Curves for sweep-net catches are based on means, over three carcasses, for each site; the curves for pitfall catches are based on means, over a maximum of four pitfall traps, for each site. For both methods, raw means were normalized to permit between-site comparisons.

numbers of calliphorids (i.e., *Phaenicia sericata* [Meigen], *Phormia regina* [Meigen]) and piophilids (i.e., *Prochyliza* sp., *Stearibia nigriceps* [Meigen]).

Comparison of accumulation rates for taxa and individuals at S2–S4, against the approximate 95% confidence limit bands for S1, show that the three sites, on a majority of days, accumulated new taxa and individuals at comparable rates. For sweep-net samples, S3 accumulated individuals at a slower rate than S1 on days 4–6 (Fig. 4B). For pitfall samples, S2 accumulated individuals faster than S1 and the other sites on days 2–5. Overall, accumulation curves for S2–S4 fell inside S1's 95% confidence bands in 60 of 72 site-days for taxa (83%) and in 56 of 72 site-days for individuals (78%), for a total of 116 (81%) site-days.

Rarefaction Analysis. A rarefaction curve plots expected number of taxa, $E_{S(n)}$, against the number of individuals, n, in a sample. Complete rarefaction curves for the cumulative sample of 2,364 and 14,456 individuals in S4 (sweep-net) and S2 (pitfall trap) samples, respectively, were drawn by standardizing (rarefying) the samples at intervals of 118 (Fig. 5A) and 206 (Fig. 5B) individuals and both are bracketed by intervals 1.96 standard deviations wide, which constitutes approximate 95% CL. In all but one comparison, sites were statistically indistinguishable in their taxonomic richness at a 95% confidence level; all four sites, however, fall within a 99% confidence interval. For the numbers of individuals in S1–S3 (Fig. 5A) and in S1, S3, S4 (Fig. 5B), the observed number of taxa for these sites are also plotted. If the number of sweep-net individuals in S1 (616), S2 (629), and S3 (247) are drawn at random from the total in S4, and if the null hypothesis is true, then the expected number of taxa

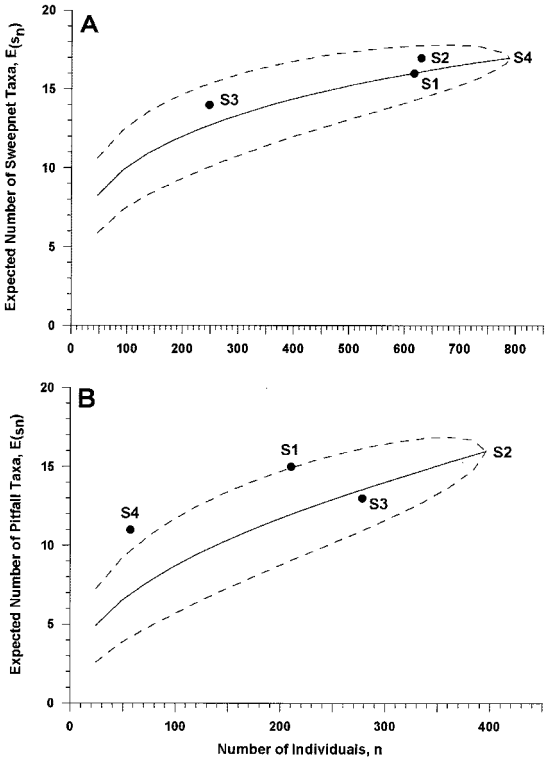

Fig. 5. Complete rarefaction curves (solid curves) and 95% CL (dashed curves) for forensically important taxa in (A) sweep-net and (B) pitfall-trap samples, based on mean abundances per site. Symbols refer to the four sampling sites described in the text, where S1 = ARF.

should fall inside the 95% CL (Fig. 5A). Likewise, if the same result holds for pitfall traps, the number of taxa for S1, S3, and S4 should all fall within the confidence interval of S2 (Fig. 5B). It can be seen that all predictions are met except that S4 in the pitfall samples falls outside the 95% confidence band (but inside the 99% limit).

The same result holds if samples from three of the four sites were reduced (rarefied) to the same number of individuals as the smallest sample taken by each method, i.e., S3 in the sweep-net data and S4 in the pitfall data. The data in Fig. 5A would show (with 95% confidence) that all four sites are statistically comparable in their taxonomic richness. For pitfall traps, the most distant site, S4, was the only exceptional site, falling slightly outside the 95% confidence band of species richness (but inside a 99% limit, Fig. 5B). This site was represented by only one pitfall trap. If we consider only minimum ranges of taxa and individuals calculated from the four pitfall traps at the other three sites, we would predict that S4 would fall within the 95% confidence band (of S2) with ranges of five taxa and 151 individuals. We conclude from these rarefaction-adjusted calculations that S1 is comparable to the other sites with respect to the density of forensically-important taxa in and around small pig carcasses sampled by sweep nets and pitfall traps.

Table 4. Spearman two-tailed rank correlations of sweep-net and pitfall trap catches for each pairwise combination of sites that included S1. Values are Spearman test results adjusted for excessive ties

Method	Site comparison		
	S1 vs. S2	S1 vs. S3	S1 vs. S4
Sweep-net samples	**0.9187**	**0.9665**	**0.8963**
Pitfall-trap samples	**0.9231**	**0.8889**	**0.7707**

Note: Numbers in bold type are significant at the 0.05 level using Bonferroni adjustment for multiple test procedures. Each method is considered independent.

If the rarefaction-adjusted estimates of species richness from the sweep nets and pitfall traps are representative of what the next study will find, that study will require a high number of replicates to have sufficient power (i.e., 90%) in detecting small differences between S1 and the other sites. To detect a significant effect of the observed magnitude, which was 1.5 species in the sweep net data after rarefying all site abundances to 247 individuals (Fig. 5A), would require 11 sweeps (instead of the three used in the current study) at an alpha level of 0.05. Likewise, a total of 64 pitfall traps per site (instead of the maximum of four used in this study) would be required to detect a significant effect of the observed magnitude of one species after rarefying all site abundances to 57 individuals (Fig. 5B).

Rank Abundance Tests. High taxonomic similarity in sweep-net and pitfall trap samples occurred across the sites. Rankings of abundance for forensically-important taxa were significantly correlated for both methods when all possible site-pairs that included *S1* were compared (Table 4). Correlations among joint taxa, although significant at all sites, were somewhat weaker in catches from pitfall traps than sweep nets. A stronger distance effect in abundance ranks occurred in samples from pitfall traps than sweep nets (Table 4). Thus, abundance ranks of forensically important taxa, sampled by sweep nets and pitfall traps, are statistically comparable across sites.

Discussion

Over the 12 days of this study, decomposition parameters were comparable across the four sites (S1–S4) in nearly all pairwise tests: carcass surface temperatures (81%, Table 1), maggot mass temperatures (88%, Table 2), remaining carcass weight (82%, Fig. 2), and periodic weight loss (83%, Table 3). Likewise, four measures of community structure, measured from sweep-net and pitfall-trap samples, showed that the four sites are comparable in the majority of pairwise tests: cumulative number of taxa (mean: 83%, Fig. 4A and C), cumulative number of individuals (mean: 78%, Fig. 4B and D), rarefaction (mean: 83%, Fig. 5A and B), and ranked abundances in joint taxa (100%, Table 4). Sweep nets and pitfall traps were nearly equally efficient in capturing high percentages of forensically important taxa (93 and 84%, respectively), as previous

studies of human remains at ARF have shown (Schoenly et al. 1996).

The fact that the null hypothesis was not rejected in the majority of these pairwise tests does not indicate that carcass decay rates or arthropod community structure are unaffected by carcass enrichment. Nonsignificant results do not imply the absence of an effect even at the experiment-wide error rate. Although the majority of pairwise site comparisons of decay rates and arthropod species richness were not statistically significant, the small sample sizes (i.e., three carcasses per site and a maximum of four pitfall traps per carcass) limited the power of this study. Additional (uncontrollable) factors that contributed to low statistical power in this study included high variability in decay rates (Fig. 2) and the joint effects of interacting (community-level) components that standard statistical procedures cannot detect (Carpenter 1989). Our posthoc power analysis of three variables (carcass weight, sweep-net taxa, pitfall-trap taxa) produced different replicate sizes (7 carcasses, 11 sweeps, and 64 traps) per site that are required to detect the observed differences in these variables (15%, 1.5 taxa, one taxon, respectively) in future experiments. To our knowledge, no previous study has incorporated this high level of sampling effort (7 carcasses, 11 sweeps, and 64 traps) at multiple sites to investigate differences in carcass decay rates and arthropod community structure. Because we failed to detect significant effects at these small magnitudes, these (unrealistically) high replicate sizes, which are unlikely to be met in future experiments, are unlikely to reveal a detectable difference in these variables.

As indicated by the shaded region in Fig. 2, pig decay rates were slower at S1 than the other sites (although not significantly so). Except for days 6–8 of the succession, decay rates remained statistically comparable among sites (Table 3). If the simultaneous presence of human and pig corpses at S1 diluted the resident sarcosaprophagous community, the raw abundances and ecostatistical results do not support it. The sampling methods show that S1 had twice the mean abundances of other sites (Fig. 5A and B), not the smallest abundances as the dilution hypothesis would predict. After such abundances were standardized to a common size by rarefaction, the four sites were found to have statistically comparable densities of sarcosaprophagous species. Likewise, Spearman rank tests showed that abundance ranks were significantly correlated across sites among the joint taxa. We suggest that variation in microclimate and carcass exposure may have affected decay rates more than biotic factors per SE. At S4, pigs were laid out on solid bedrock along a north-facing slope, whereas pigs at the other sites were placed on bare ground along west-facing slopes. Pigs at S3 were in direct sunlight longer than the others. Thus, we believe that the warming action of carcasses on rock faces and in direct sunlight may have acted to raise daily carcass temperatures and accelerate decay rates at S3 and S4, compared with those at S1 (Table 1).

Carcasses showed their largest 1-d weight loss on days 6–7 because maggot mass temperatures peaked during this same 2-d period, generating a 10–15°C increase over carcass surface temperatures. Over the 6-d period that maggot masses were observed, maggot mass temperatures remained comparable across sites on all days except day 7 (Table 2). We suggest that the cumulative and combined actions that small differences in carcass starting weight, exposure angle, and degree-days can have on biological variables, such as maggot mass temperature, may be sufficient to account for the differences seen on day 7.

In this study, collector's curves justified our use of rarefaction as a sampling model for both sweep-net and pitfall-trap catches (Fig. 1A and B). Among these sites, rarefied samples gathered by these methods can be claimed (within a 99% confidence limit) to have come from the same parent population. Diversity indices were not used in this study because, unlike rarefaction, they (typically) lack a probabilistic basis, are difficult to interpret in biologically meaningful ways, and are sensitive to both species richness and abundance of a community (Hurlbert 1971, Peet 1974, Gotelli and Graves 1996). When used alone, rarefaction is statistically weak because it ignores species identities (Simberloff 1978). However, if rarefaction is combined with similarity- or correlation-based methods (e.g., Spearman rank tests), statistical rigor and ecological understanding become jointly achievable goals.

In summary, the use of two sampling methods (sweep nets and pitfall traps) combined with probability and power testing of several independent measures of decomposition rate and community structure provided a compelling and robust test of the arthropod saturation hypothesis. Thus, carcasses at the ARF do not decay faster, nor do sarcosaprophagous taxa occur there in abnormally higher populations, or colonize carcasses faster than in surrounding sites. No evidence for impoverishment of the carrion-arthropod community was found after three decades of carcass enrichment at this site. Taken together, we conclude that this outdoor laboratory is representative of surrounding sites with respect to the sarcosaprophagous arthropod community.

Acknowledgments

This study is part of a thesis submitted by the senior author in partial fulfillment of the requirements for the M.S. degree in Entomology at the University of Missouri. R.D.H. and N.H.H. thank their fellow committee members Wayne Bailey and Robert Sites for their time, inputs and laboratory space, and all of us thank Mark Ellersieck for invaluable statistical advice on repeated measures analysis. At ARF, we thank William Bass and Lee Jantz of the Anthropology Department for providing cadaver statistics, logistical support, and unrestricted access to the UT 'Body Farm'. Elsewhere at UT and in the Knoxville area, we thank the staff of the School of Veterinary Medicine for euthanizing pigs, and John Hodges of the Plant Sciences Unit of the Agricultural Experiment Station and Roger Leitner for access to field sites. For their welcomed assistance in the field, we thank Stony Bachman,

Jason Byrd, Jennifer Gabra, Kelly Guthridge, Christine Haskell, Bob Hasty, Kathy Heinsohn, Rich Merritt, Robert Quick, Tom Rogers, Sarah Schultenover, Mark Sheperdigian, Heather Thew, John Wallace, and Gene White. We thank Robert Gbur for identification of invertebrate specimens. We thank M. Lee Goff, John Edman, and two anonymous reviewers for their many useful suggestions on the manuscript. To all these individuals and institutions we are most grateful. This study was supported by U.S. National Institute of Justice Grant 97-IJ-CX-0046 (N.H. Haskell and R.D. Hall, principal investigators).

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Received for publication 14 September 2002; accepted 10 January 2003.

Appendix. Code numbers and identities of forensically-important taxa, sorted by identification number (see Fig. 3).

ID no.	Class	Order	Family	Subfamily, genus or species	Life stage[a]
2	Insecta	Coleoptera	Cleridae	*Necrobia ruficollis*	A
7	Insecta	Coleoptera	Histeridae	?	I, A
8	Insecta	Coleoptera	Silphidae	?	A
9	Insecta	Coleoptera	Silphidae	*Nicrophorus orbicollis*	A
11	Insecta	Coleoptera	Silphidae	*Silpha americana*	A
13	Insecta	Coleoptera	Silphidae	*Silpha surinamensis*	A
14	Insecta	Coleoptera	Staphylinidae	*Creophilus maxillosus*	A
15	Insecta	Coleoptera	Staphylinidae	*Ontholestes cingulatus*	A
16	Insecta	Coleoptera	Staphylinidae	*Staphylinus badipes*	A
17	Insecta	Coleoptera	Staphylinidae	*Staphylinus cinnamopterus*	A
18	Insecta	Coleoptera	Staphylinidae	*Staphylinus maculosus*	A
19	Insecta	Coleoptera	Staphylinidae	*Staphylinus violaceus*	A
20	Insecta	Coleoptera	Staphylinidae	*Staphylinus vulpinus*	A
21	Insecta	Diptera	Calliphoridae	?	I, A
22	Insecta	Diptera	Calliphoridae	Calliphorinae	I, A
23	Insecta	Diptera	Calliphoridae	*Calliphora vicina*	A
24	Insecta	Diptera	Calliphoridae	*Chrysomya rufifacies*	I, A
25	Insecta	Diptera	Calliphoridae	Chrysomyinae	I, A
26	Insecta	Diptera	Calliphoridae	*Cochliomyia macellaria*	A
27	Insecta	Diptera	Calliphoridae	*Lucilia illustris*	A
28	Insecta	Diptera	Calliphoridae	*Phaenicia cluvia*	A
29	Insecta	Diptera	Calliphoridae	*Phaenicia coeruleiviridis*	A
30	Insecta	Diptera	Calliphoridae	*Phaenicia sericata*	A
31	Insecta	Diptera	Calliphoridae	*Phormia regina*	A
32	Insecta	Diptera	Muscidae	?	I, A
33	Insecta	Diptera	Piophilidae	?	A
34	Insecta	Diptera	Piophilidae	*Mycetaulus* sp.	A
35	Insecta	Diptera	Piophilidae	*Parapiophila* sp.	A
36	Insecta	Diptera	Piophilidae	*Prochyliza* sp.	A
37	Insecta	Diptera	Piophilidae	*Protopiophila latipes*	A
38	Insecta	Diptera	Piophilidae	*Stearibia nigriceps*	A
40	Insecta	Diptera	Sarcophagidae	*Fletcherimyia* sp.	A
41	Insecta	Diptera	Sarcophagidae	*Microcerella* sp.	A
42	Insecta	Diptera	Sarcophagidae	*Oxysarcodexia* sp.	A
44	Insecta	Diptera	Sarcophagidae	*Titanogrypa* sp.	A
45	Insecta	Diptera	Sepsidae	*Meroplius stercorarius*	A
46	Insecta	Diptera	Sepsidae	*Sepsidimorpha* sp.	A
47	Insecta	Diptera	Sepsidae	*Sepsis* sp.	A
48	Insecta	Diptera	Sphaeroceridae	?	A
49	Insecta	Diptera	Stratiomyidae	?	A
50	Insecta	Diptera	Stratiomyidae	*Allognosta* sp.	A
51	Insecta	Diptera	Stratiomyidae	*Hermetia illucens*	A
52	Insecta	Diptera	Stratiomyidae	*Mesosargus* sp.	A

[a] I, immature; A, adult stage.