ASSESSING THE INFLUENCE OF ENVIRONMENTAL STRESSORS ON ARTHROPODS AND SMALL MAMMAL PARASITE COMMUNITIES

By

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CHAPTER 1

ASSESSMENT OF THE HOST-PARASITE RELATIONSHIP AS AN *IN SITU* BIOMONITORING SYSTEM FOR EVALUATING ECOTOXICOLOGICAL RISK IN TERRESTRIAL ECOSYSTEMS

ABSTRACT- The need to examine the effects of contamination in terrestrial ecosystems has been widely recognized. The objective of this study was to assess the potential usefulness of gastrointestinal parasites of hispid cotton rats (*Sigmodon hispidus*) as a model for examining the effects of soil contamination on community level processes in terrestrial ecosystems. The gastrointestinal parasite communities of the hispid cotton rats were surveyed from 1993 to 1995 at the Oklahoma Refining Company, a Superfund Site in Caddo County, Oklahoma. Several parasite species exhibited significant interactions between treatment and season of collection, with seasonal variation generally being higher on the references sites than the contaminated sites. Significant differences in overall community structure between the contaminated and uncontaminated sites were also observed. The results of our study suggest that soil contamination could significantly alter the structure of parasite communities in terrestrial ecosystems and that parasite communities could be a useful model for assessing the effects of soil contamination in terrestrial ecosystems.

Introduction

Terrestrial ecosystems are complex, interrelated systems regulated by a multitude of biotic and abiotic processes. Although a great deal of research has been undertaken to examine the effects of anthropogenic stressors on terrestrial ecosystems, the need to establish indicators which will help to further elucidate and more accurately predict the effects of stressors and establish acceptable levels of risk in terrestrial systems has been widely recognized (Barnthouse et al., 1988; Emlen, 1989; Ma, 1994). Progress in this

regard for terrestrial ecosystems currently lags far behind that for aquatic ecosystems where a significant body of research has been accumulated on alterations in community-level processes following exposure to pollutants.

Recent attention has focused on the host-parasite relationship as a possible model for evaluating the effects of contamination on community-level processes in aquatic ecosystems. These efforts have culminated in several examples of how contaminant-induced alterations in the structure of aquatic parasite community structure can occur for many host species. A series of studies by Khan and others (Khan and Kiceniuk, 1983; Khan and Kiceniuk, 1988; Khan, 1990; Khan and Thulin, 1991) established that petrochemical contamination of marine environments significantly altered the degree of parasitism in several species of fish. Bagge and Valtonen (1996) found similar alterations in the prevalence of parasitism in fish exposed to pulp mill effluent in rivers. Increased parasitism in crayfish (*Orconectes virilis*) following lake acidification has also been reported (France and Graham, 1985). Responses of the host-parasite relationship to contamination were found to be very complex, and the responses of individual parasite species variable, depending on characteristics of the life cycle of the parasite and immune response of the host (Bagge and Valtonen, 1996). However, these studies collectively have demonstrated the feasibility of using parasite communities of a host as an indicator of anthropogenic stressors in aquatic ecosystems and suggest that they may be useful bioindicators for assessing community-level effects of contamination in terrestrial ecosystems.

Hispid cotton rats (*Sigmodon hispidus*) are an excellent model for assessing the impacts of anthropogenic stressors on host-parasite relationships in terrestrial ecosystems.

They are a common inhabitant of disturbed habitats throughout the southern United States. Habitat disturbances are a characteristic result of many forms of anthropogenic stress, especially those associated with the disposal of industrial wastes into the environment. An additional benefit of using the cotton rat is that the structure and dynamics of their parasite communities have been relatively well described in previous studies (Harkema and Kartman, 1948; Chandler, 1950; Melvin and Chandler, 1950; Melvin, 1952; Kinsella, 1974; Mollhagen, 1978; Martin and Huffman, 1980). The primary objective of our study was to evaluate the impact of contamination of soils with complex mixtures of petrochemical waste products on the structure of parasite communities of wild populations of cotton rats inhabiting disturbed tallgrass prairie ecosystems. We hypothesized that measurable alterations in prevalence, abundance, intensity, species richness, and community diversity of the gastrointestinal helminth communities of cotton rat hosts would be result from exposure to complex mixtures of soil contaminants.

Materials and Methods

Study Area

This study was conducted at the former Oklahoma Refining Company site, which is located in the city of Cyril, Oklahoma. The site has been an Environmental Protection Agency Superfund waste site since the refinery filed bankruptcy and closed in 1984. Three contaminated sites were utilized: storage pit site, land adjacent to several unlined asphalt storage pits; oil sludge trap site, land adjacent to a series of oil sludge sedimentation ponds; and soil farm site, a 3.4 ha land farming site, which was used to

process oil sludge waste materials. Two reference areas were also utilized: site one, which was located on property which was owned by the Oklahoma Refining Company but not utilized in refining operations; and site two, located on private property. Both areas were chosen based on their proximity to the contaminated sites (Both areas were located within 7.2 km of the refinery) and their ecological similarity. All grids were dominated by disturbance adapted species such as johnsongrass (*Sorghum halapense*), Sumac (*Rhus* spp.), several species of brome grasses (*Bromus* spp.), ragweed (*Ambrosia* spp.), and sagewort (*Artemisia* spp.). Although cotton rats were by far the most commonly trapped species of small mammal on all sites, we commonly trapped several other small mammal species, including *Peromyscus maniculatus*, *Peromyscus leucopus*, *Reithrodontomys fulvescens*, and *Mus musculus*.

Surveys of the major soil contaminants on the site have found heavy metals and organic waste materials from the refining process on all contaminated sites. Organics were found only in soils collected from all contaminated sites, and the primary contaminants were toluene, xylenes, pyrene, anthracene, napthalene, phenanthrene, benzo(g,h,i) perylene and benzo(a) pyrene.(Stanley Engineering Inc. 1985; USEPA Proj. No. W68439). Heavy metals were also found in soil samples from all contaminated areas. Lead and Chromium were detected from all three areas, at levels ranging from 24 to 2700 ppm for chromium and 14 to 304 ppm for lead. Arsenic was detected in soil samples from the storage pit and oil sludge trap sites, with averages of 104 ppm and 3 ppm, respectively. Aluminum, barium, zinc, and mercury were only found in soil samples from storage pits site, with levels ranging from 19 to 53,800ppm (Stanley Engineering Inc. 1985; USEPA Proj. No. W68439).

Data Collection

In order to examine possible differences in seasonal fluctuations in the helminth communities of cotton rats between contaminated and reference sites, animals were collected during five time periods (September 1993, February 1994, August 1995, April 1996, and October 1996). Cotton rats were collected by removal trapping with snap traps baited with peanut butter. Animals were subsequently weighed, sexed, necropsied, and small intestinal tracts were removed and preserved in 70% ethanol or 10% formalin. Lateral incisions were made along the intestinal tracts for gross examinations and cestodes that were found were removed. We removed the intestinal contents by scraping the mucosa with a glass slide and examined the contents with a dissecting microscope to find the smaller nematodes. Parasites were examined and taxonomically identified using lactophenol wet mounts for nematodes and borax-carmine stain mounts for cestodes; total recovery of all helminth species was attempted.

Data Analysis

The terms abundance, intensity, and prevalence were used here as defined by Margolis et al. (1982). Overdispersion was defined by Bliss and Fisher (1953) and was used to describe the frequency distributions of common species (> 25% prevalence) where a small number of the hosts harbored a large number of individuals of a parasite species. Overdispersion is indicated when the variance of a species is significantly larger than the mean abundance using a chi-square distribution. The degree of overdispersion was measured with the negative binomial k (Bliss and Fisher, 1953), and differences in

the degree of overdispersion were analyzed by using Anscombe's transformation, $Log_{10}(x + 1/2\underline{k})$ of the abundance data (Bliss and Owen, 1958).

We used a completely randomized design with repeated measures for comparisons of abundance, intensity and overdispersion. The comparisons were made using PROC MIXED (SAS, 1996) with sources of variation including treatment, site within treatment (error term for treatment), sampling date, treatment by date interaction, and the residual. A compound symmetric model was used to model the covariance structure of the repeated measurements. If the treatment by sampling date interaction was significant, simple effects of treatment were analyzed using the SLICE option for the LSMEANS statement. Satterthwait's approximation was used for calculation of the degrees of freedom of the pooled error term. If the treatment by sampling date was not significant, the main effects were analyzed with the DIFF option. Comparisons of prevalence were made utilizing Fisher's Exact Test for detection of heterogeneity among the five trapping areas during each season (PROC FREQ, SAS 1996).

Communities for each host population were described by measures of diversity, mean species richness, and similarity. Diversities were calculated by using the complement of Simpson's index, 1- D (Krebs, 1989). Comparisons of mean species richness and species diversity among treatments were made using analysis of variance with repeated measures as described previously. Similarities in species composition and relative abundances of helminth communities among cotton rat populations were calculated using Horn's index (Horn, 1966) and relationships between communities were depicted using single-linkage cluster diagrams (Krebs, 1989). Statistical significance for all hypothesis tests was set *a priori* at $\underline{P} \leq 0.05$.

Results

Helminth Fauna and Prevalence

All but four of the 340 cotton rats which we collected were infected with at least one species of helminth (Tables 1 and 2). Three species of cestodes (*Raillietina sigmodontis*, *Schizotaenia sigmodontis*, and *Hymenolepis dimunuta*) and four species of nematodes (*Protospirura muris*, *Longistriata adunca*, *Syphacia sigmodontis*, and *Strongyloides sigmodontis*) were recovered (Table 2). *Longistriata adunca*, a trichostrongylid nematode, was the most prevalent species, infecting 316 of the 340 cotton rats. *Schizotaenia sigmodontis* was the most prevalent cestode in the study, infecting 49% of the cotton rats which we surveyed.

Prevalences among helminth populations within each season were extremely variable, and no consistent patterns of difference between the three contaminated and two reference sites were evident for most species (Figs. 1 and 2). *Longistriata adunca* was found in over 80% of the animals on all sites in the first four seasons, but prevalence dropped below 60% in animals from the storage pit site in October 1995 (Fig. 2). *Hymenolepis dimunuta* was not observed in September 1993 or February 1994, and *Syphacia sigmodontis* was not observed in October 1995; all other species were observed in every sampling period. There were three seasons in which the prevalence of a helminth species on both reference sites was significantly different from any of the contaminated sites. *Syphacia sigmodontis* was more prevalent ($P \leq 0.05$) in cotton rats collected from the reference sites than the sludge trap and soil farm sites in April 1995. In September 1994 prevalence of infection with *Strongyloides sigmodontis* was greater ($P \leq 0.05$) in cotton rats from the reference sites than the storage pit and sludge trap sites, where this

species was not recovered from any host animals examined. *Protospirura muris* was five times more prevalent on the reference sites than the sludge trap and soil farm sites ($P \leq 0.05$) in October 1995. Although consistent differences in prevalences from one season to another between contaminated and reference sites were lacking for all species, the differences in prevalences of *Strongyloides sigmodontis* among the contaminated study sites over the course of the survey were noteworthy. Prevalences of *Strongyloides sigmodontis* varied widely on the reference sites across seasons, ranging from 10 to 50%, while remaining consistently low (0 to 15%) in cotton rats from all of the contaminated sites throughout the survey.

Helminth Abundance

Total abundances of cestodes in cotton rats in our survey were influenced significantly by season of collection ($P \leq 0.0001$) but not treatment. The differences in cestode abundances were primarily due to low overall cestode numbers in February 1994 and April 1995 compared to the fall collections. The abundances of *Schizotaenia sigmodontis* infections were influenced by season of collection and by treatment (Table 3). *Schizotaenia sigmodontis* was twice as abundant in host animals on the reference sites than the contaminated sites ($P < 0.005$). The influence of season of collection ($P < 0.01$) on the abundance of *Schizotaenia* was primarily due to the 50% decrease in abundances of the species during October 1995 compared to other seasonal collections (Table 2). Abundances of *Raillietina sigmodontis* infections were not influenced by treatment ($P > 0.05$), but were significantly influenced by season of collection ($P \leq 0.0001$), and there was also a significant season by treatment interaction ($P < 0.05$; Table 3). Analysis of the

simple effects indicated that there was a significantly larger variation in abundances *Raillietina sigmodontis* on the contaminated sites, with higher abundances in the Fall collections and lower abundances in April 1995 and February 1994, when no infected animals were recovered from the contaminated sites (Table 2). Although a similar seasonal pattern of higher fall abundances was present in animals from the reference populations, variation in abundances of *Raillietina sigmodontis* on the reference sites was considerably lower and was only significantly ($P \leq 0.05$) different from other seasons when it increased in October 1995 (Table 2). Abundances of infection with *Hymenolepis dimunuta* were significantly different across seasons ($P < 0.0005$). There was also a significant treatment by season interaction ($P < 0.05$) due to higher abundances of *Hymenolepis dimunuta* in cotton rats from the reference sites in October 1995 (Table 2).

Remarkable differences were also observed in seasonal abundances of several nematode species infecting cotton rats in our study. Abundances of *Protospirura muris* showed significant seasonal fluctuations ($P \leq 0.0001$), with a strong treatment by date interaction ($P < 0.005$). Seasonal changes in abundances of *Protospirura muris* infections in cotton rats from reference sites varied almost 8-fold across seasons, while significant seasonal changes in abundances on contaminated sites were not apparent (Table 2). Similar to *Protospirura muris*, abundances of *Syphacia sigmodontis* infections were significantly different across seasons ($P < 0.05$) , with a significant season by treatment interaction ($P < 0.01$). Reference host populations had significantly greater fluctuations in abundances of *Syphacia sigmodontis* infections across seasons compared to host populations from contaminated sites. Abundances of *Syphacia sigmodontis* from reference host populations were lower in February 1994 and April 1995 than September

1994 and October 1995, while abundances of infections in host animals from contaminated sites were only different from other seasonal collections in September 1994 (Table 2). The large mean abundance of *Syphacia sigmodontis* infections from hosts on the contaminated sites in September 1994 was due to a few heavily infected animals, including one host which harbored over 9000 individuals. Abundances of *Strongyloides sigmodontis* were also influenced by season ($P \leq 0.01$), with a strong treatment by season interaction ($P < 0.005$). Analysis of the simple effects showed that seasonal abundances of *Strongyloides sigmodontis* infections also varied widely in host populations on reference sites, peaking in September 1993 and 1994; abundances remained very low (less than 0.1 worms per host) in cotton rat populations from contaminated sites across all seasons (Table 2).

Abundances of *Longistriata adunca* changed significantly with season ($P \leq 0.0001$), but were not influenced by treatment ($P > 0.05$). Abundances of infection with *Longistriata adunca* were especially low in February 1994 and October 1995 relative to the other seasons (Table 2). Overall abundances of nematodes were significantly influenced by season ($P < 0.05$) but not treatment.

Helminth Intensities

Intensity of infection with *Hymenolepis dimunuta*, *Syphacia sigmodontis*, *Strongyloides sigmodontis*, and total nematodes showed no relationship ($P > 0.05$) to treatment or season (Table 5). Intensity of infection with *Raillietina sigmodontis* was significantly influenced by season of collection ($P \leq 0.05$), primarily because of a dramatic increase in intensity of infection during October 1995 compared to other

seasons. Overall intensity of cestode infections was also influenced by season of collection ($P \leq 0.0001$), with infections tending to be more intense in fall than winter and spring collections (Table 4). *Longistriata adunca* exhibited a strong seasonal fluctuations ($P \leq 0.0001$) in intensities but was not influenced by treatment ($P > 0.05$; Table 4). *Protospirura muris* infections showed seasonal changes ($P \leq 0.0001$) with a significant season by treatment interaction ($P < 0.05$). Intensities of infection with *Protospirura muris* fluctuated as much as four-fold from one season to the next in reference host populations, while no apparent seasonal variability was seen in contaminated sites (Table 4).

Helminth Distribution

Only *Longistriata adunca* and *Protospirura muris* had prevalences > 25% in all seasons for analysis of overdispersion. Other species were not suitable for this type of analysis because they fell below 25% prevalence during some or all seasons. The distribution of *Longistriata adunca* and *Protospirura muris* changed by season ($P \leq 0.0001$) but showed no indication that the degree of overdispersion for either species was influenced by treatment ($P \geq 0.05$; Table 6).

Helminth Community

Mean species richness of gastrointestinal parasite communities within host populations was significantly influenced by treatment ($P \leq 0.05$; Table 7). Host animals on the reference sites consistently supported a greater average number of helminth species than those from contaminated sites in all seasonal collections (Table 7). Mean

species richness also differed across seasons ($P < 0.05$), being significantly higher in September 1994 than in April and October 1995 (Table 7). Diversity (complement of Simpson's index) did not appear to change seasonally or differ significantly between treatments ($P > 0.05$), ranging from 0.232 to 0.603 (Table 7).

Comparisons of the similarity of species composition and abundances among helminth communities showed that the reference sites were more apt to cluster together than with the contaminated sites, especially in February 1994, September 1994, and April 1995 (Fig. 3). Although reference site 1 was most similar to the sludge pits site in October 1995, the two reference sites clustered together at > 97% similarity. In September 1993 the three contaminated sites were clustered together, but the reference sites were not.

Discussion

Studies of aquatic ecosystems have demonstrated the potential of pollution to considerably disrupt the host-parasite relationship in many species. Khan and Kiceniuk (1983) observed a significant decrease in gastrointestinal parasite prevalence and intensity of infection in both Atlantic Cod (*Gadus morhua*) and winter flounder (*Sterningophorus furciger*) following exposure to crude oil. In a later study Khan and Kiceniuk (1988) observed an increase in monogean gill parasitism in Atlantic cod following chronic exposure to crude oil. In this case the increase in parasitism was attributed to a high incidence of contamination-induced gill lesions. Skinner (1982) made similar observations on several fish species from Biscayne Bay, Florida. An increase in burdens of some gill parasites has been observed in populations of *Rutilis rutilis* when

exposed to pulp mill effluents (Bagge and Valtonen, 1996). Tuuha et al.(1992) observed that some species of gill parasites were more prevalent in roach which inhabited pulp mill effluent contaminated lakes, while others were more prevalent in hosts from uncontaminated lakes. In addition to the contamination-induced gill lesions described above, immunosuppresion may make hosts more vulnerable to parasitism by suppressing key components of the immune response (Bagge and Valtonen, 1996). Also, direct impacts of contamination on free-living stages of parasites could potentially result in either decreased or increased parasitism, depending on the sensitivity of the parasite species (Tuuha et al., 1992).

Although largely ignored in the past, there is evidence that anthropogenic stressors do disrupt host-parasite relationships in terrestrial ecosystems. Boggs et al. (1991) observed significant alterations in the structure of helminth communities of cotton rats residing in habitats exposed to triclopyr and tebuthiuron herbicides. They observed lower prevalences of *Raillietina* sp. and *Syphacia sigmodontis* in host populations following herbicide application. Boggs et al. (1991) also noted that *Longistriata adunca* was more overdispersed in summer than in winter in cotton rat populations on herbicide-treated areas, but not in populations from reference sites. In a similar study Boggs et al. (1990) observed a decrease in abundance of *Obeliscoides cuniculi* infections in cottontail rabbit (*Sylvilagus floridanus*) populations residing in areas altered by prescribed fire and herbicide application compared to those collected from reference areas.

All three cestodes and one of the nematode species recovered from cotton rats in this study have complex life cycles which require arthropod intermediate hosts and all

four of these species demonstrated a significant treatment effect or treatment by season interaction. It seems plausible that any impacts (positive or negative) from soil contamination on the availability of intermediate hosts could play a profound role in the regulation of these helminth species in their hosts. Dipterans (flies), coleopterans (beetles), dictyopterans (cockroaches), and hymenopterans (ants) have been found to be important intermediate hosts for some species of *Raillietina* (Ackert, 1922; Horsefall, 1938; Georgi and Georgi, 1990). *Schizotaenia sigmodontis* requires soil-dwelling oribatid mites (suborder oribatida) as intermediate hosts (Melvin, 1952). The intermediate stages of *Hymenolepis dimunuta* most commonly develop in fleas and flour beetles (Georgi and Georgi, 1991). Spirurids such as *Protospirura muris* utilize coleopterans and orthopterans as intermediate hosts (Yamaguti, 1961).

The sensitivities of several taxonomic groups of arthropods to exposure to chemical contaminants have been demonstrated. Stamou and Argyropoulou (1995) observed that oribatid mite communities expose to complex mixtures of pollution in urban areas underwent dramatic changes in structure, often losing sensitive species altogether, while other species were higher in density in the contaminated areas. Other soil microarthropods have also been shown to be sensitive to soils contaminated with metals (Parmelee et al., 1993), herbicides (Badjedo and Akinyemiju, 1993) and pesticides (Perry et al., 1997). Adverse consequences of aquatic contamination on intermediate host populations and consequential alterations in prevalence and intensity of parasite infections in definitive hosts have been documented (Overstreet and Howse, 1977).

The other three nematode species we recovered all have direct life cycles, although there are still some notable life cycle differences among them. *Longistriata*

adunca and *Syphacia sigmodontis* are both shed by the host directly into the environment, where they are transmitted to new hosts by ingestion of eggs or larvae. The results of our study suggest that seasonal fluctuations in abundances of infection with *Syphacia sigmodontis* were potentially influenced by contamination, but that abundances of *Longistriata adunca* were more significantly influenced by seasonal factors and not by contamination.

Species of the genus *Strongyloides* have a unique heterogenic life cycle, with alternation of free-living and parasitic female generations. The males are not parasitic, residing in a free-living form in the soil. The parasitic filariform larvae enter the host by direct penetration of the skin (Melvin and Chandler, 1950). It seems probable that this life cycle would expose the free-living generations directly to the contaminants in the soils, which could have a negative effect on the densities of free-living generations and on the availability of males, which would explain the consistently low prevalences and abundances of parasitic females we observed on contaminated areas. The sensitivity of *Strongyloides* to changes in other soil factors, such as variations in pH, temperature, and moisture, have also been demonstrated (Primvati, 1958).

Overall effects of petrochemical soil contamination on the structure (species composition and abundances) of the parasitic communities were evident in the single-linkage cluster analysis of similarity indices. Also, host animals inhabiting the reference sites harbored a richer parasitic fauna than hosts residing on the contaminated sites, suggesting that contamination negatively impacted community structure. These results suggest that soil contamination was an important contributor in shaping helminth communities of the cotton rat host populations. Much of the variability in the degree of

impact of contamination was probably due to differences in the life cycles of the individual parasite species. Given the differences in populations and overall community structure which we observed, host-parasite relationships seem to have promise as a biomonitoring tool for the assessment of community-level effects of contamination in terrestrial ecosystems.

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Table I: Summary of cotton rat trapping data from Oklahoma Refining Co. superfund waste site, Caddo county, Oklahoma, September 1993 to October 1995.

Date	Population	n	Males	Females
September 1993	Reference site 1	16	6	10
	Reference site 2	15	7	8
	Contaminated site 1	17	8	9
	Contaminated site 2	18	7	11
	Contaminated site 3	17	10	7
	Total	83	39	44
February 1994	Reference site 1	13	9	4
	Reference site 2	12	7	5
	Contaminated site 1	15	8	7
	Contaminated site 2	12	5	7
	Contaminated site 3	18	5	13
	Total	70	34	36
September 1994	Reference site 1	15	7	8
	Reference site 2	13	8	5
	Contaminated site 1	16	6	10
	Contaminated site 2	15	5	10
	Contaminated site 3	15	10	5
	Total	74	36	38
April 1995	Reference site 1	12	3	9
	Reference site 2	12	6	6
	Contaminated site 1	5	1	4
	Contaminated site 2	13	7	6
	Contaminated site 3	13	7	6
	Total	55	24	31
October 1995	Reference site 1	11	5	6
	Reference site 2	12	6	6
	Contaminated site 1	12	7	5
	Contaminated site 2	11	7	4
	Contaminated site 3	12	6	6
	Total	58	31	27

Table 2. Means and standard errors of abundances of gastrointestinal helminth infections in cotton rats collected from the reference and contaminated sites on the Oklahoma Refining Company site, September 1993 to October 1995.

		September 1993		February 1994		September 1994		April 1995		October 1995	
Helminth species	Treatment	Mean	S E	Mean	S E	Mean	S E	Mean	S E	Mean	S E
Raillietina sigmodontis	Reference	0.50	0.20	0.24	0.17	1.32	0.59	0.08	0.04	3.65	1.26
	Contaminated	4.50	1.02	0	0	1.28	0.40	0.61	0.43	5.23	1.52
Schizotaenia sigmodontis	Reference	2.30	0.61	1.84	1.32	1.21	0.35	1.58	0.51	0.91	0.31
	Contaminated	0.98	0.27	1.06	0.20	1.61	0.36	1.13	0.30	0.37	0.35
Hymenolepis dimunuta	Reference	0	0	0	0	1.00	0.83	0.21	0.21	2.09	1.18
	Contaminated	0	0	0	0	0.22	0.08	0.03	0.03	0.09	0.06
Protospirura muris	Reference	3.52	0.72	11.88	2.43	4.75	1.05	1.50	0.63	7.96	1.90
	Contaminated	4.14	0.83	3.72	0.82	4.59	0.77	2.16	0.82	4.61	1.14
Longistriata adunca	Reference	91.77	32.17	24.80	4.05	46.46	8.22	63.17	13.81	24.35	5.62
	Contaminated	28.82	5.78	14.15	2.07	25.50	4.80	25.39	6.47	10.05	3.71
Syphacia sigmodontis	Reference	82.58	80.55	76.04	30.01	2.04	1.54	57.33	122.72	0	0
	Contaminated	0.24	0.21	41.55	32.72	282.17	205.22	2.87	1.98	0	0
Strongyloides sigmodontis	Reference	3.87	3.10	0.24	0.13	2.54	1.63	0.41	0.21	0.48	0.21
	Contaminated	0.06	0.03	0.09	0.04	0.02	0.02	0.06	0.06	0.08	0.04

Table 3. F and P values generated by ANOVA for repeated measures comparisons of main effects of treatment, date, and interactions on species abundances for the intestinal helminth from cotton rats on reference and contaminated sites on the Oklahoma Refining Company waste site, Caddo County, Oklahoma, August 1993 to October 1995.

		Raillietina sigmodontis		*Schizotaenia sigmodontis*		*Hymenolepis dimunuta*		*Protospirura muris*		*Longistriata adunca*		*Syphacia sigmodontis*		*Strongyloides sigmodontis*	
Effect	Degrees of freedom	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Treatment	1	2.26	0.2248	11.45	0.0008	1.84	0.2693	2.31	0.2259	4.88	0.1139	0.32	0.6131	9.50	0.0543
Date	4	13.36	0.0001	3.81	0.0048	5.65	0.0002	6.08	0.0001	8.74	0.0001	2.71	0.0300	3.38	0.0099
Treatment x date	4	2.98	0.0195	1.80	0.1286	3.01	0.0184	4.16	0.0027	0.80	0.5244	3.46	0.0087	4.30	0.0021

Table 4. Means and standard errors of intensities gastrointestinal helminth infections in cotton rats collected from the reference and contaminated sites at the Oklahoma Refining Co. Superfund waste site, Caddo County,Oklahoma, from September 1993 to October 1995.

		September 1993		February 1994		September 1994		April 1995		October 1995	
Helminth species	Treatment	Mean	S E	Mean	S E	Mean	S E	Mean	S E	Mean	S E
Railiettina sigmodontis	Reference	1.67	3.28	2.00	4.28	4.63	2.78	1.00	5.25	9.33	2.66
	Contaminated	7.71	1.67	.	.	4.54	2.22	4.75	3.73	9.63	1.91
Schizotaenia sigmodontis	Reference	3.54	0.56	4.30	0.45	2.62	0.56	2.82	0.49	2.33	0.67
	Contaminated	2.47	0.46	2.00	0.40	3.52	0.44	2.00	0.49	2.17	0.76
Hymenolepis dimunuta	Reference	.[a]	.	.	.	9.33	3.95	5.00	6.84	8.00	2.79
	Contaminated	.	.	.	.	1.28	2.42	1.00	6.84	1.50	4.84
Protospirura muris	Reference	4.54	0.82	15.63	2.66	6.65	1.24	3.89	0.98	9.15	2.06
	Contaminated	7.14	1.14	6.73	1.20	6.59	0.90	3.94	1.36	7.65	1.67
Longistriata adunca	Reference	94.83	30.85	26.95	4.09	46.46	8.22	64.13	13.81	26.67	5.91
	Contaminated	31.72	6.04	15.11	2.14	26.07	4.88	27.14	6.80	14.08	4.99
Syphacia sigmodontis	Reference	365.75	355.58	118.81	43.73	11.40	7.96	96.64	45.64	.	.
	Contaminated	4.00	3.00	130.20	101.00	1081.67	762.76	29.67	14.52	.	.
Strongyloides sigmodontis	Reference	17.14	13.23	2.00	0.00	4.73	1.63	2.00	0.63	2.20	0.37
	Contaminated	1.00	0.00	1.00	0.00	1.00	.	2.00	.	1.00	0.00

[a] = not testable (only one or no animals infected)

Table 5. F and P values generated by analysis of variance for repeated measures for the main effects of treatment, date and interactions for intensities of gastrointestinal helminth infections from cotton rats collected from the refernce and contaminated areas on the Oklahoma Refining Company site, Caddo County, Oklahoma, August 1993 to October 1995.

		Raillietina sigmodontis		*Schizotaenia sigmodontis*		*Hymenolepis dimunuta*		*Protospirura muris*		*Longistriata adunca*		*Syphacia sigmodontis*		*Strongyloides sigmodontis*	
Effect	Degrees of freedom	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Treatment	1	0.60	0.4416	0.89	0.3917	3.62	0.0766	0.44	0.5537	4.58	0.1210	0.02	0.8923	1.52	0.2251
Date	4	1.79	0.1332	2.86	0.0283	0.03	0.9685	6.17	0.0001	6.16	0.0001	0.25	0.9085	0.33	0.8567
Treatment x date	4	0.89	0.4699	1.43	0.2404	0.03	0.9727	4.31	0.0023	0.67	0.6147	2.08	0.1114	0.42	0.7912

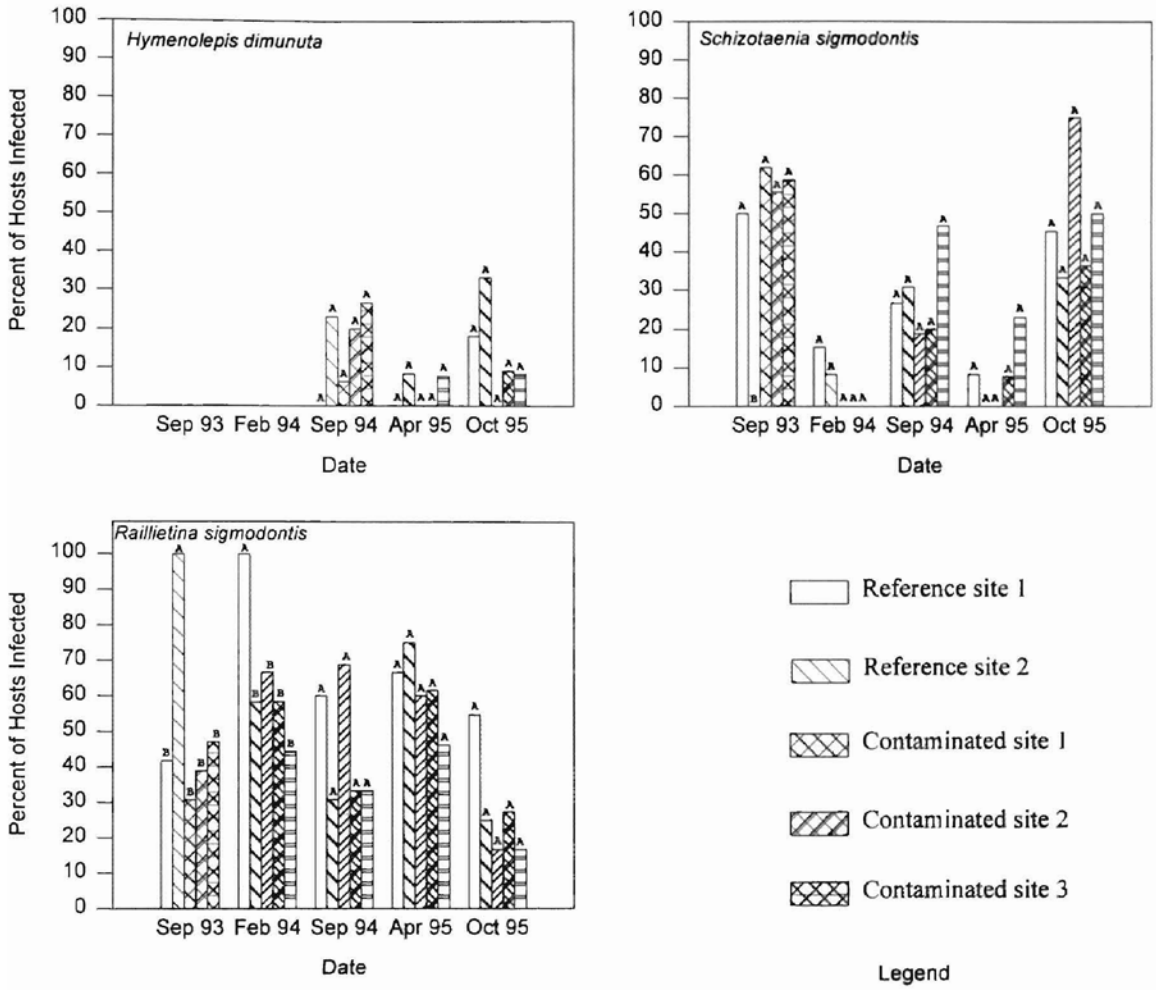
Table 6. Mean $\underline{k}$ values and standard errors for degree of overdispersion of the helminth species *Protospirura muris* and *Longistriata adunca* between contaminated and reference sites at the Oklahoma Refining Co. Superfund waste site, Caddo County, Oklahoma, September 1993 to October 1995. Results were non-significant for both species which were tested.

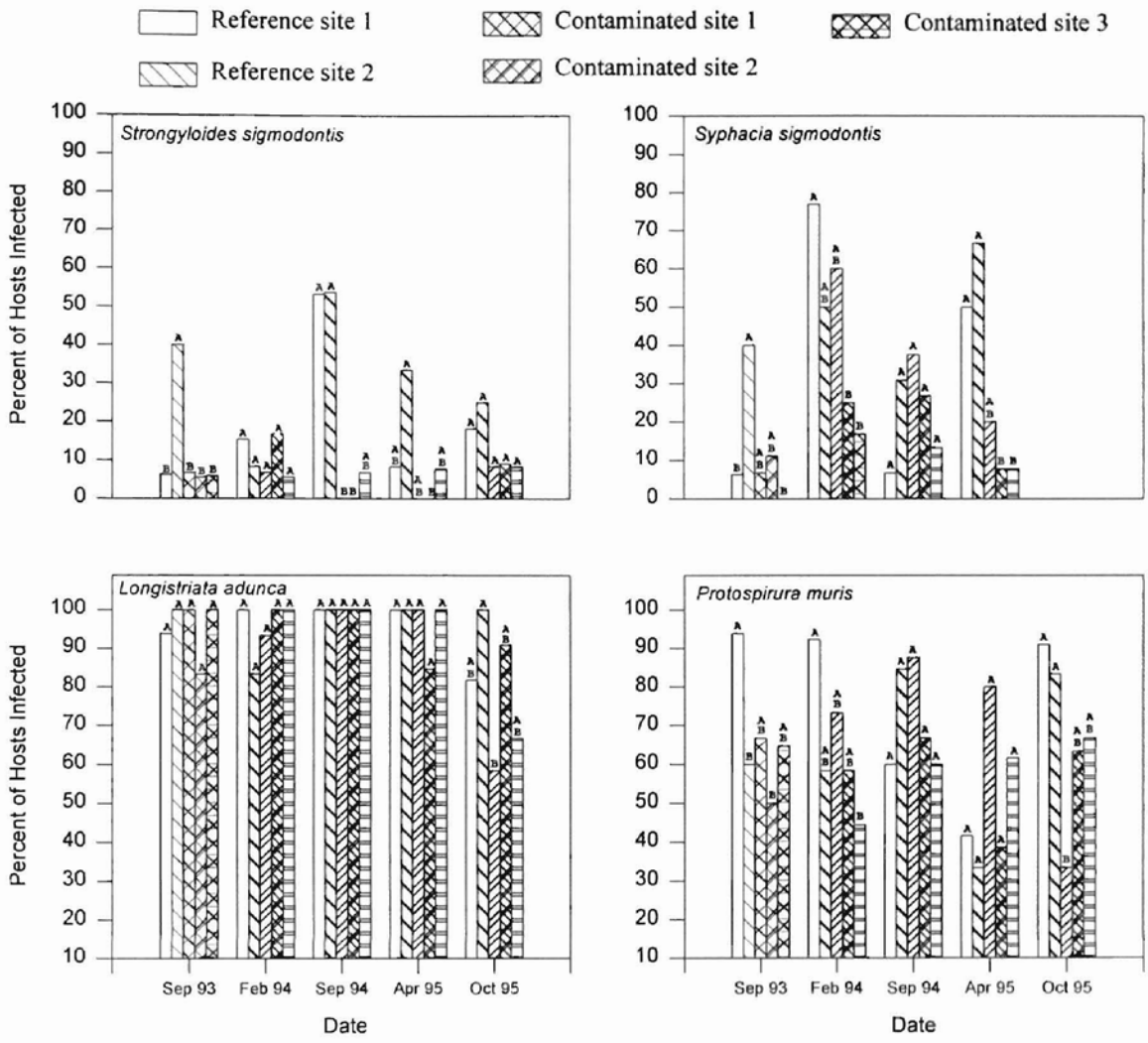
	Longistriata adunca				*Protospirura muris*			
	Reference		Contaminated		Reference		Contaminated	
Date	Mean	SE	Mean	SE	Mean	SE	Mean	SE
September 1993	0.46	0.37	0.70	0.09	1.10	0.32	0.55	0.10
February 1994	1.71	0.70	1.32	0.20	1.39	0.63	0.53	0.25
September 1994	1.22	0.09	0.65	0.09	1.13	0.61	1.07	0.65
April 1995	3.48	1.77	1.35	0.44	0.54	0.07	1.60	1.15
October 1995	0.87	0.30	0.52	0.17	0.94	0.30	0.57	0.05

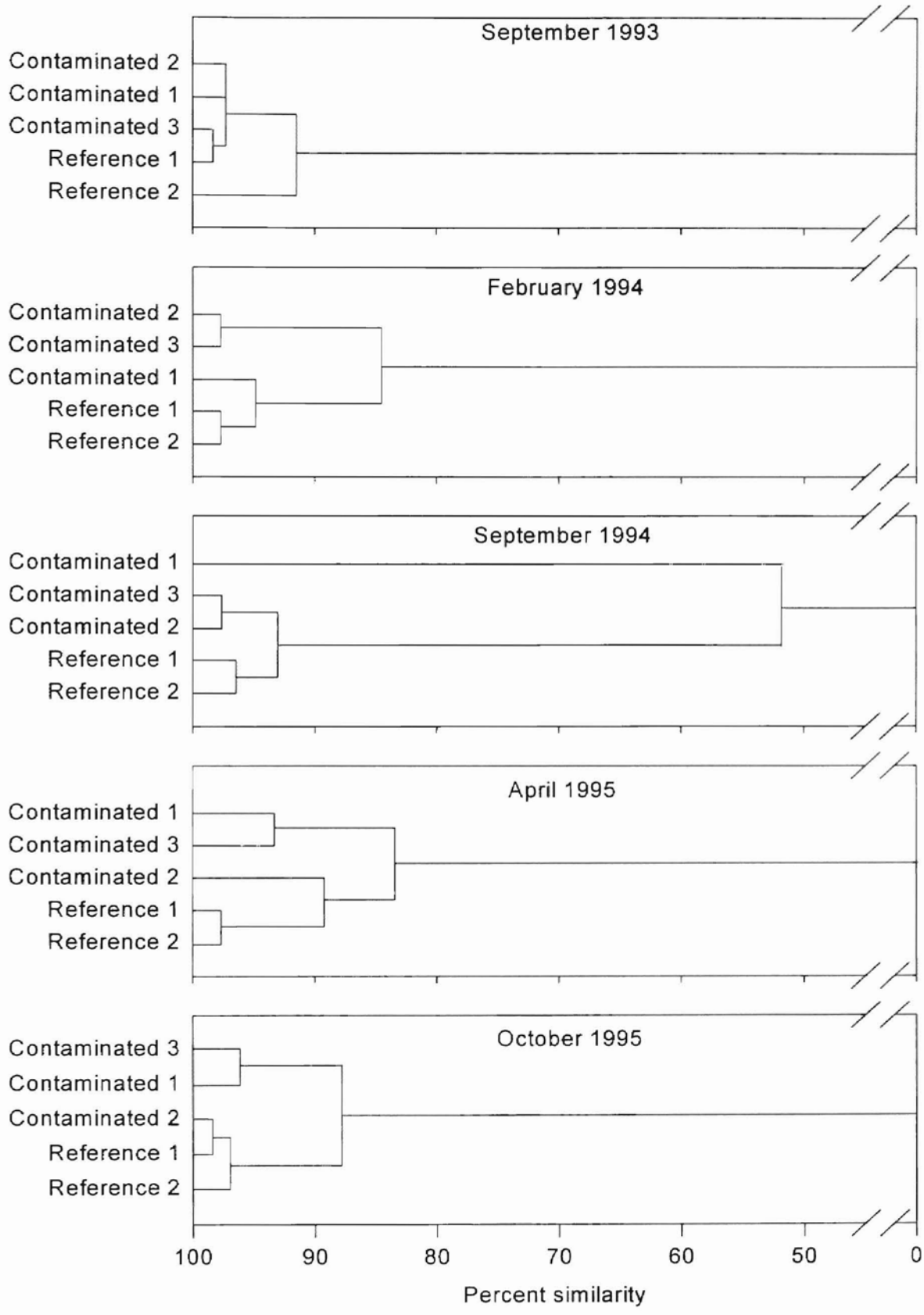
Figure 1: Seasonal prevalences of cestode species infecting cotton rats from three contaminated and two reference sites on the Oklahoma Refining Co. Superfund waste site, Caddo County, Oklahoma, August 1993 to October 1995. Different letters above the bars denote significant differences in prevalence among study populations within each season (calculated with Fisher's Exact Test, $P \leq 0.05$).

Figure 2: Seasonal prevalences of nematode species infecting cotton rats from three contaminated and two reference sites at the Oklahoma Refining Co. superfund waste site, Caddo County, Oklahoma, August 1993 to October 1995. Different letters above the bars denote significant differences in prevalence among study populations within each season (calculated with Fisher's Exact Test, $P \leq 0.05$).

Figure 3: Single-linkage cluster diagrams depicting seasonal changes in similarity (Horn, 1966) of helminth community composition and abundances in cotton rat hosts from three contaminated and two reference sites at the Oklahoma Refining Co. Superfund waste site, Caddo County, Oklahoma, August 1993 to October 1995.


Hymenolepis dimunuta
Schizotaenia sigmodontis
Raillietina sigmodontis
Percent of Hosts Infected
0
10
20
30
40
50
60
70
80
90
100
Sep 93
Feb 94
Sep 94
Apr 95
Oct 95
Date
Reference site 1
Reference site 2
Contaminated site 1
Contaminated site 2
Contaminated site 3
Legend


Reference site 1
Reference site 2
Contaminated site 1
Contaminated site 2
Contaminated site 3
Strongyloides sigmodontis
Syphacia sigmodontis
Longistriata adunca
Protospirura muris
Percent of Hosts Infected
Percent of Hosts Infected
Sep 93
Feb 94
Sep 94
Apr 95
Oct 95
Date
Date


September 1993
Contaminated 2
Contaminated 1
Contaminated 3
Reference 1
Reference 2
February 1994
Contaminated 2
Contaminated 3
Contaminated 1
Reference 1
Reference 2
September 1994
Contaminated 1
Contaminated 3
Contaminated 2
Reference 1
Reference 2
April 1995
Contaminated 1
Contaminated 3
Contaminated 2
Reference 1
Reference 2
October 1995
Contaminated 3
Contaminated 1
Contaminated 2
Reference 1
Reference 2
100
90
80
70
60
50
0
Percent similarity

CHAPTER 2

ASSESSMENT OF THE HOST-PARASITE RELATIONSHIP OF COCCIDIA AS AN *IN SITU* BIOMONITORING SYSTEM FOR EVALUATING ECOTOXICOLOGICAL RISK IN TERRESTRIAL ECOSYSTEMS

Introduction

Terrestrial systems are complex in nature, and populations and communities are regulated by many processes. Parasitism has been suggested to play an important role in the dynamics of some host populations through both direct mortalities, declines in fitness, and increased host susceptibility to predation (Holmes 1995; Murray et al. 1997). The coccidia are ubiquitous parasite group which have been found in virtually every major vertebrate group, including mammals, birds, reptiles, amphibians, and fish (Levine and Ivens 1965; Pellerdy 1965). Eimerians are a common group of the coccidia which have a direct life cycle and are transmitted by the ingestion of infective sporulated oocysts by host species. Following ingestion, they penetrate the epithelium of the gastrointestinal tract and reproduce (Fuller 1996a). Temporal fluctuations in populations of many coccidian species have been observed as a result of extrinsic and intrinsic factors such as weather (Fuller 1996a; Wilber et al. 1994) and host immune status (Fuller et al. 1995; Fuller 1996b). Parasite species-dependent traits, such as thickness of oocyst walls, sporulation time, and patency period have also been implicated as factors shaping coccidian populations in host populations (McQuiston 1984). The effects of anthropogenic factors on the coccidian community structure has not been well studied, although some researchers have found effects, and have suggested that coccidia might be a useful model community for assessment of soil contamination effects (Wilbur et al. 1994).

The primary objective of our study was twofold: to assess diferences in coccidian prevalence among cotton rat (*Sigmodon hispidus*) populations inhabiting reference sites

and areas contaminated with complex mixtures of petrochemicals, and also to examine the temporal and spatial changes in the structure of these coccidian communities. We were particularly interested in evaluating the potential influence of season, age, sex, and other biotic factors (interspecific competition or facilitation, host immune status) in the regulation of coccidian communities and interpopulation variation in infection rates in this host.

A series of predictions were examined to evaluate some of the potential intrinsic and extrinsic environmental factors that regulate the structure of coccidian communities in host populations of cotton rats. First, we hypothesized that if competition or facilitation between coccidian species occurs, it should be indicated by either fewer (competition) or more (facilitation) double infections than would be expected by random chance (Reduker and Dusznyski 1985; Fuller 1996a). We also hypothesized that host-acquired immunity to coccidia would be indicated by a greater prevalence of infection in juveniles than adults, assuming that the probability of exposure does not change with age (Wilber et al. 1994; Fuller 1996a). A third factor that could potentially explain variation in infection rates within populations of cotton rats is endocrine differences between sex and reproduction classes. Because of androgen-induced immunosuppression in males, we hypothesized that infection rates in males would be greater than in females (Fuller 1996a). Similarly, we hypothesized that pregnancy-induced immunosuppression (Tizard 1992) would result in greater rates of infection in pregnant than non-pregnant females (this also assumes equal likelihood of exposure to infection between pregnant and non-pregnant females).

Materials And methods

Study area

This study was conducted at the former Oklahoma Refining Company site, which is located in the city of Cyril, Oklahoma. The site has been an Environmental Protection Agency Superfund waste site since the refinery filed bankruptcy and closed in 1984. Three contaminated sites were utilized: storage pits, land adjacent to several unlined asphalt storage pits; sludge trap site, land adjacent to a series of oil sludge sedimentation ponds; and soil farm site, a 3.4 ha land farming site, which was used to process oil sludge waste materials. Two reference areas were also utilized: reference one, which was located on property which was owned by the Oklahoma Refining Company but not utilized in refining operations; and reference two, located on private property. Both areas were chosen based on their proximity to the contaminated sites (Both areas were located within 7.2 km of the refinery) and their ecological similarity. All grids were dominated by disturbance adapted species such as johnsongrass (*Sorghum halapense*), Sumac (*Rhus* spp.), several species of brome grasses (*Bromus* spp.), ragweed (*Ambrosia* spp.), and sagewort (*Artemisia* spp.).

Surveys of the major soil contaminants on the site have found heavy metals and organic waste materials from the refining process on all contaminated sites. Organics were found only in soils collected from all contaminated sites, and the primary contaminants were toluene, xylenes, pyrene, anthracene, napthalene, phenanthrene, benzo(g,h,i) perylene and benzo(a) pyrene.(Stanley Engineering Inc. 1985; USEPA Proj. No. W68439). Heavy metals were also found in soil samples from all contaminated areas.

Lead and Chromium were detected from all three areas, at levels ranging from 24 to 2700 ppm for chromium and 14 to 304 ppm for lead. Arsenic was detected in soil samples from the storage pit and oil sludge trap sites, with averages of 104 ppm and 3 ppm, respectively. Aluminum, barium, zinc, and mercury were only found in soil samples from storage pits site, with levels ranging from 19 to 53,800ppm (Stanley Engineering Inc. 1985; USEPA Proj. No. W68439).

A mark-recapture census was used to establish densities of host populations prior to the study as described previously by McMurry (1993). All study populations supported high densities of cotton rats, ranging from a low of 20-40 animals per hectare in the winter of 1991 to peak densities of ca. 100-120 cotton rats per hectare in the summer of 1991 (McMurry 1993). Ten species of small mammals were captured during this census, including 9 species of rodents and 1 species of insectivore. Although cotton rats were the dominant small mammal on our study sites, we also collected *Peromyscus leucopus*, *Peromyscus maniculatus*, *Reithrodontomys fulvescens*, *Mus musculus*, *Microtus ochregastor*, *Microtus pinetorum*, *Neotoma floridana*, *Chaetodipus hispidus* and *Blarina hylophaga*.

Data collection

Cotton rats were sampled over four collection periods (February 1994, August 1995, April 1995, and October 1995). Cotton rats were obtained by removal trapping using standard snap traps baited with peanut butter. The rats were placed on ice and transferred to the laboratory within 48 hours, where they were weighed, sexed, and necropsied for assessment of reproductive status, general condition, and harvest of

coccidia from the gastrointestinal tract. This animal research was approved by the Oklahoma State University Institutional Animal Care and Use Committee as protocol number 329.

Contents of the large intestine of each animal were removed by lateral incision of the large intestine and scraping of the mucosa with a glass slide. The contents were placed in petri dishes with a thin layer of 2% (w/v) potassium dichromate solution. Fecal material was macerated and mixed thoroughly with the potassium dichromate and kept at room temperature (20° to 25° C) for 13-15 days. The long sporulation period was necessary for *Eimeria tuskegeensis* (Barnard et al. 1974). Samples were then placed in individually labeled, screw-top vials and stored in a refrigerator at 4° C until they could be examined for oocysts.

In order to examine fecal samples for oocysts, storage vials were mixed and 5 ml of solution were removed and strained through two layers of cheesecloth. Oocysts were concentrated from the strained material by sucrose flotation onto glass coverslips and examined with a light microscope at 100x magnification (Duszynski et al. 1982). Individual oocysts were measured and identified to species using a calibrated ocular micrometer at 1000x magnification under immersion oil. Two replicate flotations were prepared for each animal and the presence of each species found in the flotation was recorded.

Data analysis

Pairwise comparisons of prevalence of infection between groups (female vs. male, adult vs. juvenile) and among seasons and individual populations within seasons were

analyzed using Fisher's Exact Test (PROC FREQ, SAS 1993) for each species of eimerian. Because of the low prevalences of *Eimeria tuskegeensis* and *E. roperi*, comparisons were restricted to *E. sigmodontis* and *E. webbae*. The expected number of double infections was calculated by multiplication of the proportions of host animals infected with *E. sigmodontis* and those infected with *E. webbae* with the sample size (n) as described by Fuller (1996). The expected and actual numbers of double infections were compared using Fisher's Exact Test. Statistical significance for all tests was set a priori at $P \leq 0.05$. One-tailed tests were used for the specific hypotheses that prevalence of infection in one group would be greater than in another and two-tailed results were utilized for testing differences among and within seasons.

Results

Species Richness and Prevalence

Two hundred and twenty six cotton rats were captured over the four collection periods (Table 1), and five species of coccidian parasites were recovered (Table 2). One identified *Isospora* sp. was found in only 1.3% of the hosts. Coccidian infections were common throughout the year, with an overall prevalence of 72%. Although the largest proportion of cotton rats (49.8%) were observed to harbor a singlespecies, the occurrence of infections with two species was also common (20.3%). Triple infections were rare (only 2.2%) and no host was found to harbor all four of the *Eimeria* species. *Eimeria sigmodontis* was the most prevalent coccidian recovered from cotton rats in our study, being found in twice as many hosts as was the second most common species, *Eimeria*

webbae (Table 2). *Eimeria tuskegeensis* and *E. roperi* were both uncommon, with overall prevalences of less than 15% (Table 2).

Differences Among Populations

The prevalences of coccidian parasites among the five populations were highly variable within seasons. Significant differences in the prevalence of *E. sigmodontis* infections were found among sites in both February and September 1994. In February, the prevalence of infection in reference two was only a third of that in the contaminated sites ($P < 0.05$). In September, cotton rats from sludge traps and land farm had a higher prevalence of infection than those from both reference populations ($P < 0.05$); no infections were observed in animals from reference two in the February collection. There were no significant differences in the prevalence of *E. sigmodontis* infections among populations sampled in April and October 1995 (Fig. 1).

The prevalence of *Eimeria webbae* infections was also variable among populations. Cotton rats collected from storage pits in February 1994 had a significantly higher prevalence of infection with *E. webbae* than those collected from reference site 1, which had no infected animals ($P < 0.05$). Reference 2 also had no animals infected with *E. webbae*. Unlike *E. sigmodontis*, significant differences in the prevalence of *E. webbae* were observed in April 1995. The prevalence of *E. webbae* infections in cotton rats from reference population 1 was six times higher than the prevalence in sludge pits site ($P < 0.025$). No significant differences in the prevalence of *E. webbae* infections among host populations were observed in either September 1994 and October 1995.

Seasonal Dynamics of Infection

Seasonal fluctuations in prevalence were observed in three of the four *Eimeria* species that were identified in cotton rat hosts. The prevalence of infections with *E. sigmodontis* in cotton rat populations was twice as abundant ($P < 0.05$) in February 1994 and April 1995 compared to other collection dates (Fig. 2). In contrast, *E. webbae* infection rates were more ($P < 0.05$) abundant in September 1994 and October 1995 than April 1995 (Fig. 2). Prevalences of *E. roperi* and *E. tuskegeensis* infections were almost uniformly low throughout the study (the exception was a large increase in the prevalence of *E. tuskegeensis* in October 1995) and showed no discernible seasonal patterns; infections varied from 1% to 10% (Fig. 2). Distribution of uninfected animals across seasons largely remained constant (21-22%). The only exception was a two-fold increase in percentage of uninfected animals during September, 1994, which was significantly higher than in February 1994 (Table 3).

Biotic Factors Influencing Infection

We found no evidence that the frequency of hosts with dual infections of *E. sigmodontis* and *E. webbae* differed from what was expected by chance ($P > 0.05$; Table 4), indicating that neither positive nor negative associations between these two species occurred in large intestines of cotton rats from our study populations. Comparisons of prevalence between male and female hosts revealed no differences ($P > 0.05$) for either *E. sigmodontis* or *E. webbae* (Fig. 3), indicating no support for the hypothesis that hormone-induced immunosuppression in males increased infection rates. Predictions of age-related acquired resistance to infections of cotton rats by these parasites were not supported by

comparisons of infection rates between adults and juveniles ($P > 0.05$). Also, comparisons of infection rates between pregnant and non-pregnant females for both *Eimeria* species during the September 1994 and October 1995 breeding seasons showed no support ($P > 0.05$) for the hypothesis of pregnancy-induced immunosuppression as a factor in infection rates of these species (Fig. 4).

Discussion

The survey of coccidia of 34 cotton rats by McAllister et al. (1993) in north Texas was insufficient to provide meaningful comparisons of prevalence with our study populations in Oklahoma. McAllister et al. (1993) did observe four of the same species of *Eimeria* that we noted in our study, but they did not observe the *Isospora* sp. that was found both in our study and also by Barnard et al.(1974) in cotton rats in Alabama. Also, we did not observe the unidentified *Eimeria* sp. that was found in cotton rats in both Texas and Alabama. Neither of these two species are considered to be normal parasites of *Sigmodon hispidus*, since Barnard et al. (1974) were unable to reproduce infections in cotton rats from exposure in the laboratory.

Our findings were in agreement with those of Barnard et al. (1974), who documented that *E. sigmodontis* was the most prevalent coccidian in two populations of cotton rats in Alabama. They observed that 84.5% of the cotton rats they examined were infected with *E. sigmodontis* (Barnard et al. 1974). Host populations in Oklahoma showed considerable seasonal variation in rates of infection with *E. sigmodontis*, ranging from 25.0 to 71.4% , with an overall prevalence of 51.3%. Prevalence of infection with

E. webbae in cotton rats from Alabama was 46.6%, which was comparable to the maximum prevalence observed in cotton rats from Oklahoma (40.6%). Prevalence of *E. webbae* in Oklahoma was highest in host populations in the fall. *E. tuskegeensis* was the least common species found in cotton rats in Alabama (prevalence of 22.8%), whereas the prevalence in our study varied from 1.9 to 43.8% , with an overall prevalence of 10.6%. The most notable difference in coccidia between our study and that of Barnard et al. (1974) was the prevalence of *E. roperi*. Cotton rats in Alabama populations were commonly infected (40.9%) with this species, but it was uncommon in our populations (maximum seasonal prevalence of only 6.7%).

The differences in prevalences among the contaminated and reference sites were not consistent, and did not conclusively support the hypothesis that soil contamination had an effect on prevalence of coccidian infections. The only other study we found on the effects of soil contamination on coccidia concerned radon contamination. Wilber et al (1994) observed that coccidia oocysts collected from feces of northern pocket gophers failed to sporulate under laboratory conditions, while those collected from a nearby uncontaminated area sporulated normally. They did not observe differences in prevalence of coccidia between the sites, and suggested that the gophers inhabiting the radon rich area were most likely ingesting oocysts while foraging in radon poor areas. It may be that prevalence of coccidia infection is not a sensitive indicator of environmental contamination, but few studies have been done to either confirm or dispute this. A larger study, with more frequent sampling and larger sample sizes, is needed to address these issues..

Some of the observed differences in prevalence among the *Eimeria* species can be explained by species-dependent factors, such as sporulation time and wall thickness of the oocysts. Both of these factors are believed to play a role in oocyst survival in the external environment (McQuiston 1984; Fuller 1996a). The sporulation time of *E. sigmodontis* has been found to be approximately 2 days, whereas *E. webbae* and *E. roperi* oocysts take 3-4 days and *E. tuskegeensis* oocysts take 11-12 days (Barnard et al. 1974). The prevalence of three of the four species of *Eimeria* that infected cotton rats in our study fit a pattern based on sporulation time. *Eimeria sigmodontis*, the most prevalent species, was documented by Barnard et al. (1974) as having the shortest sporulation time among all of the populations of *Eimeria* they studied. In contrast, *E. tuskegeensis*, documented as having a very long sporulation period by Barnard et al. (1974), was much less common in our study than *E. Sigmodontis*. *Eimeria webbae*, which was observed to have an intermediate sporulation time by Barnard et al. (1974), was intermediate in its prevalence in our host populations. However, *E. roperi*, which was documented by Barnard et al. (1974) as having an intermediate sporulation time, was the least prevalent species in our study, suggesting that other factors may have affected the prevalence of this species. The average wall thickness of *E. roperi* oocysts is 1.7 um, compared to walls that are 1 um thick for both *E. webbae* and *E. sigmodontis* (Barnard et al. 1974). This morphological feature would suggest that *E. roperi* should be more resistant to environmental desiccation than other species and consequently should persist in the environment longer, therefore allowing it to infect larger numbers of cotton rats. Given that this did not observe greater infection rates, neither of these traits adequately explained the low prevalence of *E. roperi* in cotton rats from Oklahoma. One factor that

could have influenced the prevalence of *E. roperi* in cotton rats in our study was the mean patency period (the period during which a host animal sheds oocysts into the environment). According to Barnard et al. (1974) the mean patency period for *E. roperi* was 2.9 days, the shortest of the four *Eimeria* species which we recovered. The short patency period could have limited the distribution of *E. roperi* oocysts in the habitat and the total number of oocysts produced by an infection compared to the other species we recovered.

Similarities between our expected and actual frequency of dual infections with *E. sigmodontis* and *E. webbae* in cotton rats from Oklahoma were in agreement with the findings of Reduker and Duszynski (1985) for wood rats (*Neotoma albigula*) and Fuller (1996a) for deer mice (*Peromyscus maniculatus*) for other species of coccidia. Although facultative associations have been documented for some parasites, such as species of *Plasmodium* (Schall and Bromwich 1994), our results do not support the hypothesis that interspecific competition or facilitation occurs between the common eimerians in the intestinal tracts of cotton rats. It has been suggested that eimerians avoid competition by spatial partitioning of the gastrointestinal tract (Khysen 1972; Fuller 1996a).

Comparisons of age, gender, and reproductive classes suggested that acquired resistance does not play a significant role in the regulation of prevalence of infections with *E. sigmodontis* or *E. webbae* in cotton rat populations. This conclusion is tempered by the fact that comparisons between pregnant and non-pregnant females were constrained by small sample size in our study. Due to the nature of Fisher's Exact Test, we were unable to calculate statistical power in this instance. It is also important to emphasize that very young juveniles (< 40 g body mass) were not sampled in our study

since they do not readily enter traps, thus biasing any attempt to evaluate differences between age classes. Although we did not find evidence of acquired immunity in cotton rats, other mammalian species have been shown to acquire immunity to *Eimeria* infections. The lack of evidence for immunity playing a role in regulating the structure of coccidia communities in cotton rats could be a reflection of differences in the degree of host animal exposure or variability in immunogenicity among *Eimeria* species, although this was not explored in our study. It is also possible that intensity or severity of infection with coccidia might be a more sensitive indicator than prevalence for evaluating the role of immunity in shaping coccidia communities. For example, Fuller et al. (1995) found variation in oocyst output between different *Eimeria* species in laboratory populations of deer mice. Deer mice developed complete immunity (no oocysts produced) to *E. delicata* after a single inoculation, while inoculation with *E. arizonensis* elicited only partial immunity (> 90% decrease in oocyst output over the course of infection) at the same dosage. Acquired immunity to *Eimeria* infection has also been observed in field populations of deer mice exposed to *Eimeria arizonensis* (Fuller 1996b).

Given the dynamic seasonal changes in prevalence of *E. sigmodontis* and *E. webbae* infections in cotton rat populations we monitored, involvement of host immunity cannot be ruled out as a possible contributing factor. It is possible that host-dependent factors such as seasonal changes in nutrition influenced coccidian population dynamics. This was demonstrated by Buffenstein and Yahav (1991) who observed a 93% decline in numbers of protozoa in naked mole rats (*Heterocephalus glaber*) following a 2.4-fold increase in dietary fiber and a 2.5-fold decrease in dietary starch. It is also possible that abiotic factors such as changes in temperature or photoperiod could have altered host

resistance to coccidia infections. The influence of climatic and photoperiodic alterations in the environment on immune system function has been observed in several mammalian species (Houstek and Holub 1994; Nelson et al. 1996) and avian species (Dabbert et al. 1997). Nelson et al. (1996) found that decreasing photoperiods had a negative impact on immune function in prairie voles (*Microtus ochrogastor*). It is also possible that changes in prevalence resulted from alterations in the microenvironment, that could affect sporulation directly. Graat et al. (1994) demonstrated that interval to sporulation and time of survival of oocysts could be altered significantly by modifying temperature in a laboratory experiment. Wilber et al. (1994) found that prevalence of *Eimeria* infections in a natural population of Townsend's ground squirrels (*Spermophilus townsendii*) decreased in a year following drought. The results of our survey suggest that dynamic seasonal patterns in rates of infection do exist in populations of *E. sigmodontis* and *E. webbae*, but most likely do not exist in *E. roperi* or *E. tuskegeensis*.

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Table 1. Sex and age characteristics of cotton rat hosts collected seasonally from soutwestern Oklahoma, February 1994 to October 1995.

Collection date	Host population	n	Males	Females	Adults	Juveniles
February 1994	1	12	9	3	4	8
	2	15	8	7	7	8
	3	13	5	8	7	6
	4	17	5	12	5	12
	5	8	6	2	3	5
	Total	65	33	32	26	39
September 1994	1	15	7	8	11	4
	2	16	6	10	9	7
	3	16	5	11	13	3
	4	13	9	4	13	0
	5	13	8	5	12	1
	Total	73	35	38	58	15
April 1995	1	11	3	8	9	2
	2	5	1	4	4	1
	3	13	7	6	10	3
	4	13	7	6	13	0
	5	12	6	6	7	5
	Total	54	24	30	40	14
October 1995	1	11	5	6	10	1
	2	12	6	6	12	0
	5	11	6	6	10	1
	Total	34	17	17	32	2

Table 2. Overall prevalence of four species of coccidia from hispid cotton rats collected in Caddo County, Oklahoma, February 1994 to October 1995.

Species	Number infected	Number examined	Percent prevalence
Eimeria sigmodontis	116	226	51.3
Eimeria webbae	79	226	35.0
Eimeria tuskegeensis	24	226	10.6
Eimeria roperi	19	226	4.4
Isospora sp.	3	226	1.3

Table 3: Seasonal distribution of coccidia infections in hispid cotton rats collected from southwestern Oklahoma from February 1994 to October 1995. The number of individuals harboring one, two, or three species of coccidia are shown; the percentage of the total is shown in parentheses.

Collection date	n	Uninfected	One species	Two species	Three species
February 1994	65	14(21.5)[b]	35(53.8)[a]	16(24.6)[a]	0(0)[a]
September 1994	73	29(39.7)[a]	29(39.7)[a]	13(17.8)[a]	2(2.7)[a]
April 1995	54	12(22.2)[ab]	28(55.6)[a]	10(18.5)[a]	2(3.7)[a]
October 1995	35	8(22.9)[ab]	19(54.3)[a]	7(20.0)[a]	1(2.9)[a]

[a,b,ab] Significant differences in prevalence (Fisher's Exact Test, $P \leq 0.05$) are denoted by different letters.

Table 4. Observed number of cotton rats infected with either *Eimeria sigmodontis, Eimeria webbae* or both species,the expected number of double infections based on chance (calculated as the sum of the proportions of animals infected with *E. sigmodontis* and *E. webbae* multiplied by the sample size)is also shown (Fuller (1996a)).

Collection date	Observed					
	n	*E. sigmodontis*	*E. webbae*	Both	Expected	P[a]
February 1994	65	48	13	11	11	1.000
September 1994	73	21	30	10	10	1.000
April 1995	54	39	13	11	9	0.805
October 1995	34	8	13	2	3	1.000

[a]Results of Fisher's Exact Test comparison of the observed and expected number of double infections.

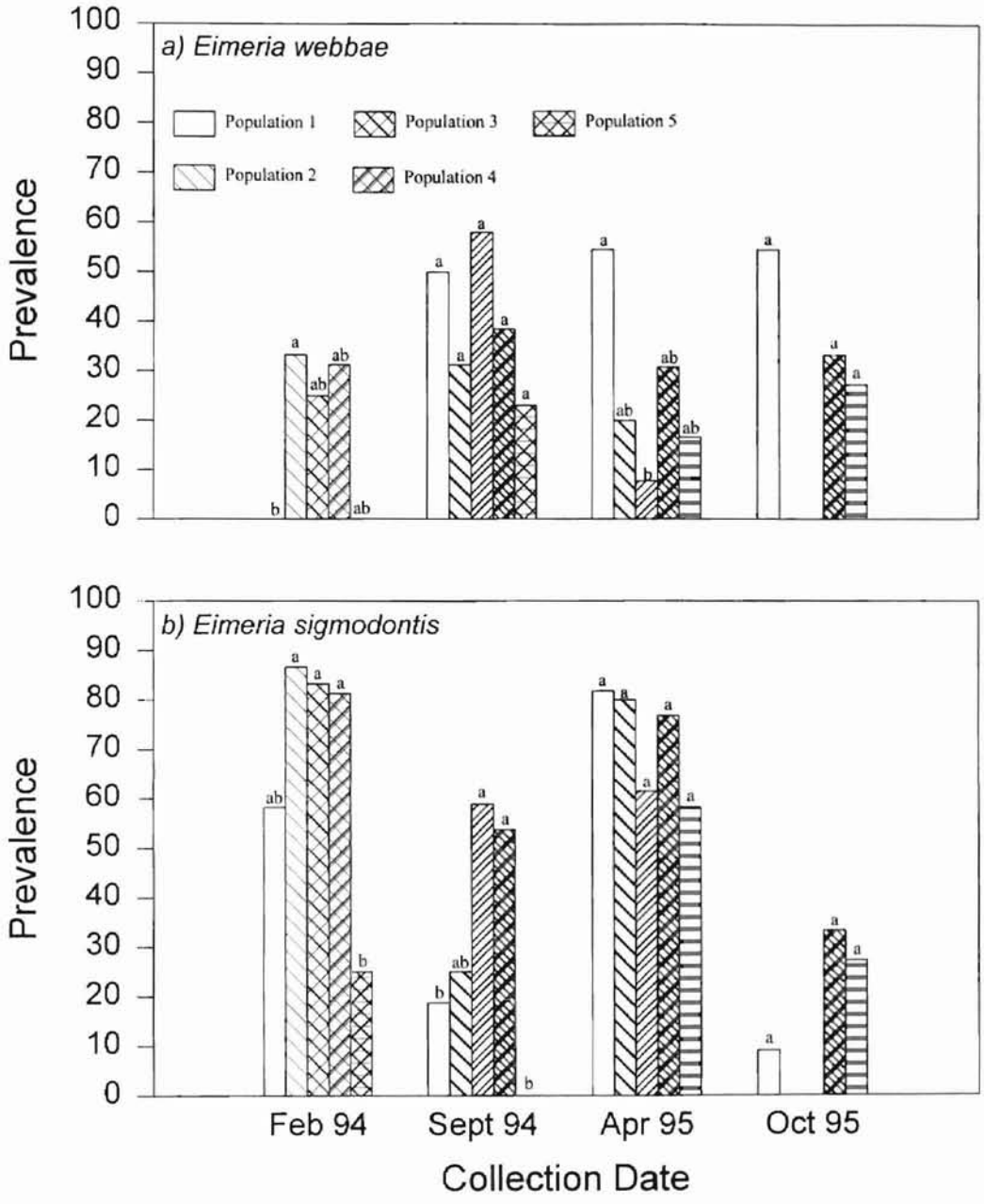
Fig.1: Seasonal prevalences of *Eimeria sigmodontis* and *Eimeria webbae* infections in hispid cotton rats collected from southwestern Oklahoma, February 1994 to October 1995. Significant differences in prevalence ($P < 0.05$, Fisher's Exact Test) among populations within each season are denoted by different letters. Populations 2 and 3 were not sampled in October 1995.

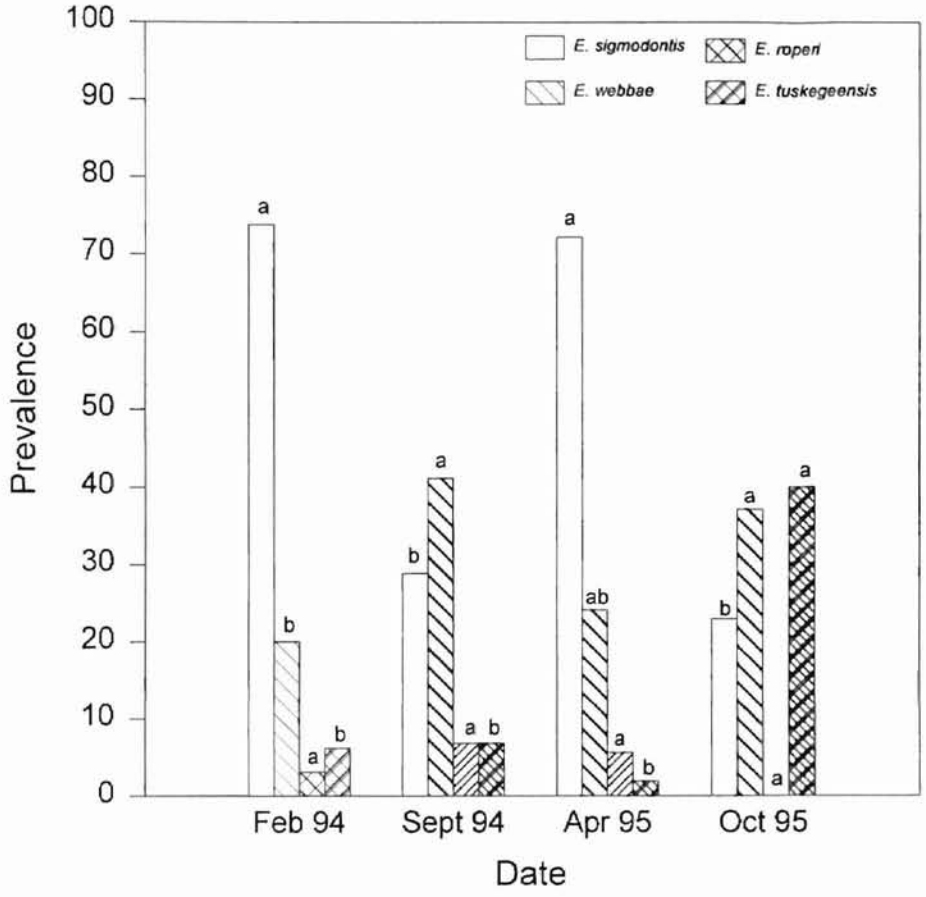
Fig. 2: Seasonal prevalences of four species of *Eimeria* infections in hispid cotton rats collected from southwestern Oklahoma, February 1994 to October 1995. Significant differences in prevalence ($P < 0.05$, Fisher's Exact Test) among populations across seasons are denoted by different letters. Populations 2 and 3 were not sampled in October 1995.

Fig. 3: Seasonal prevalences of *Eimeria sigmodontis* and *Eimeria webbae* infections in male and female hispid cotton rats collected from southwestern Oklahoma, February 1994 to October 1995. Prevalences of infection were similar ($P > 0.05$, Fisher's Exact Test) between gender classes for both *E. sigmodontis* and *E. webbae*.

Fig. 4: Seasonal prevalences of *Eimeria sigmodontis* and *Eimeria webbae* infections in adult and juvenile hispid cotton rats collected from southwestern Oklahoma, February 1994 to October 1995. Prevalences of infection were similar ($P > 0.05$, Fisher's Exact Test) between age classes for both *E. sigmodontis* and *E. webbae*.

Fig. 5: Seasonal prevalences of *Eimeria sigmodontis* and *Eimeria webbae* infections in pregnant and non-pregnant female hispid cotton rats collected from southwestern Oklahoma, February 1994 to October 1995. Prevalences of infection were similar ($P > 0.05$, Fisher's Exact Test) between reproductive classes for both *E. sigmodontis* and *E. webbae*.


a) Eimeria webbae
Population 1
Population 3
Population 5
Population 2
Population 4
Prevalence
100
90
80
70
60
50
40
30
20
10
0
b) Eimeria sigmodontis
Feb 94
Sept 94
Apr 95
Oct 95
Collection Date


E. sigmodontis
E. roperi
E. webbae
E. tuskegeensis
Prevalence
100
90
80
70
60
50
40
30
20
10
0
a
b
a
b
b
a
a
b
a
ab
a
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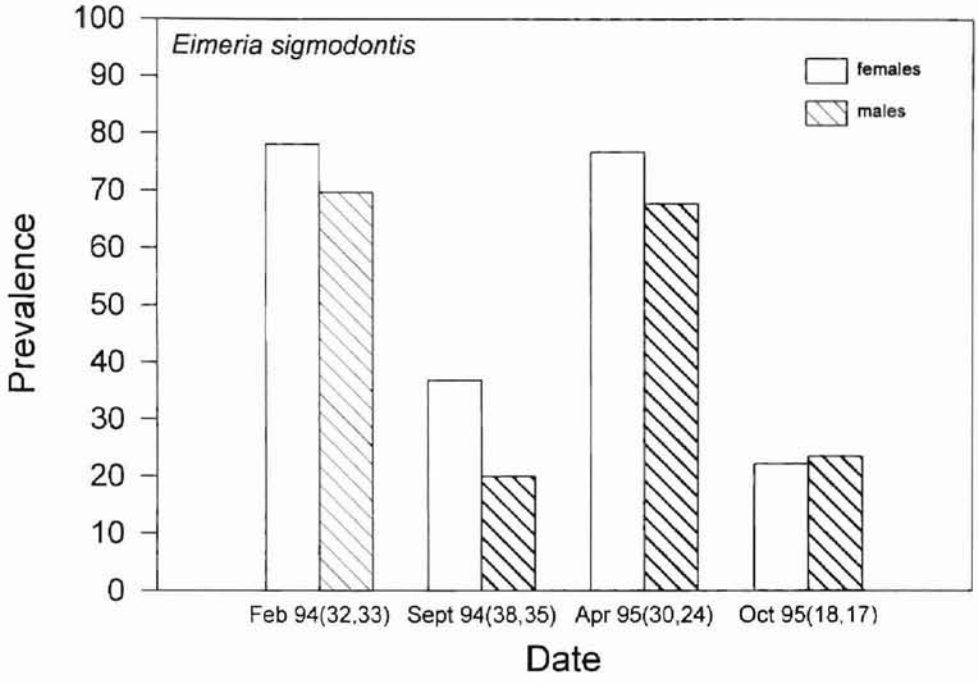
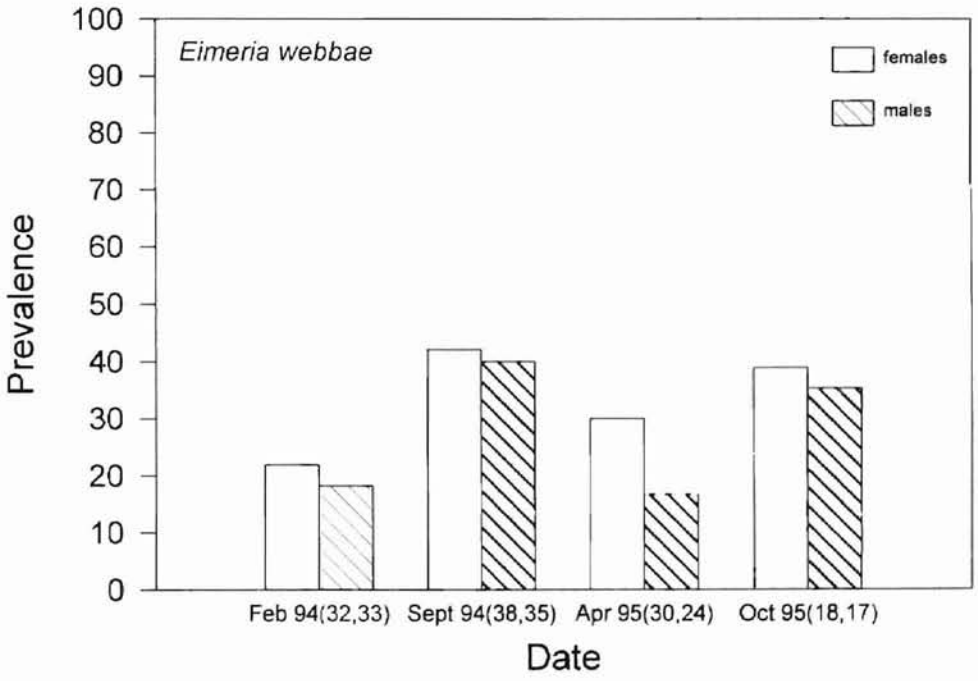
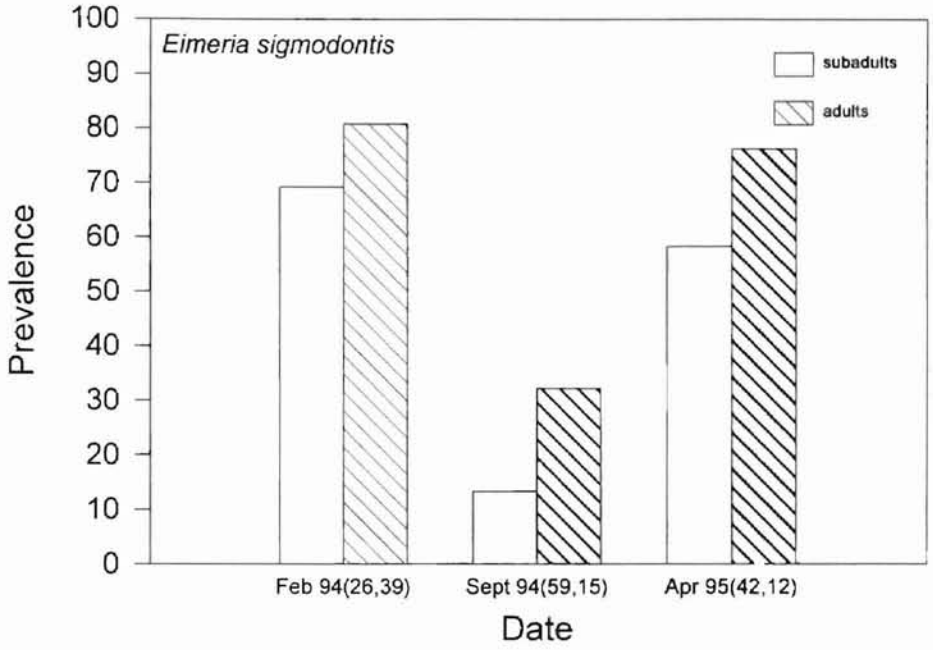
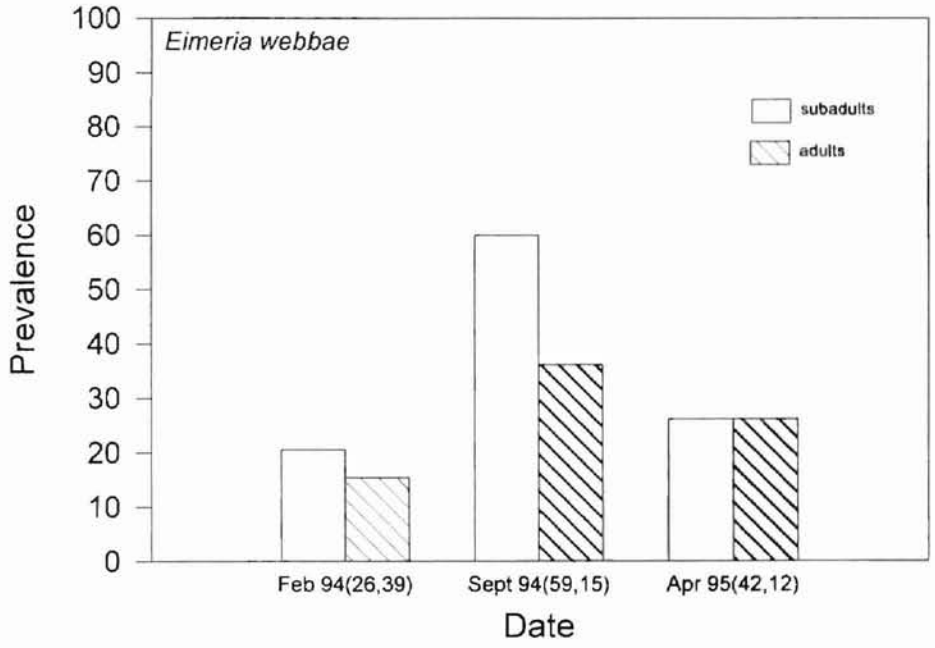
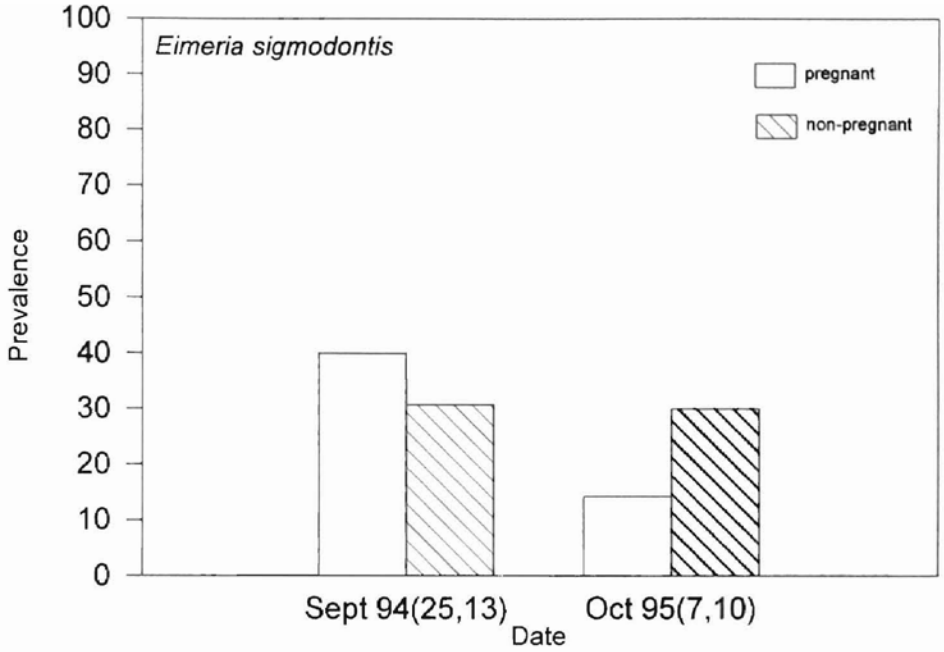
b
a
a
Feb 94
Sept 94
Apr 95
Oct 95
Date

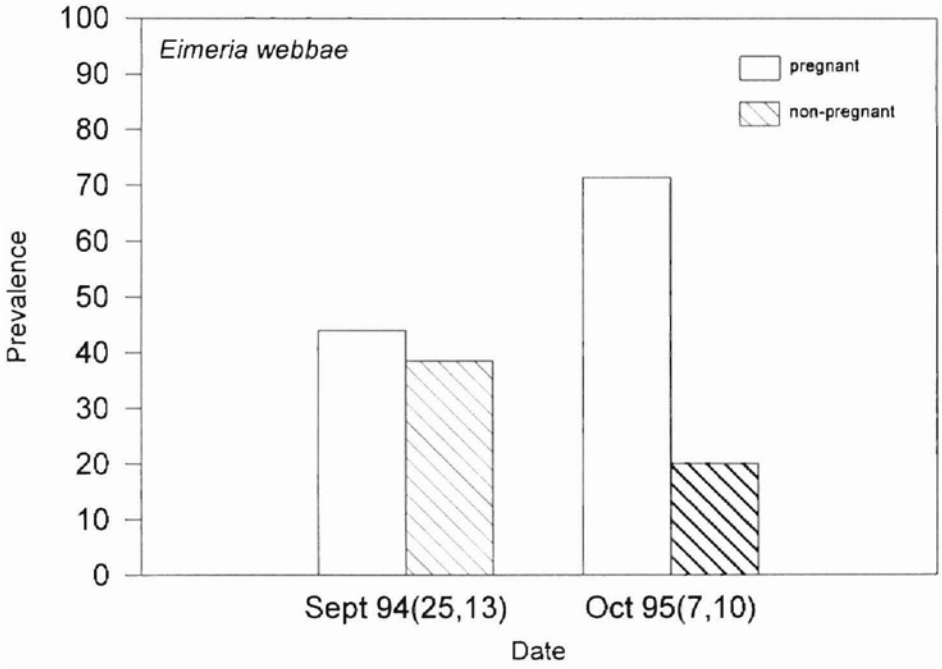

Eimeria sigmodontis
females
males
Prevalence
100
90
80
70
60
50
40
30
20
10
0
Feb 94(32,33)
Sept 94(38,35)
Apr 95(30,24)
Oct 95(18,17)
Date


Eimeria webbae
females
males
Prevalence
100
90
80
70
60
50
40
30
20
10
0
Feb 94(32,33)
Sept 94(38,35)
Apr 95(30,24)
Oct 95(18,17)
Date


Eimeria sigmodontis
subadults
adults
Prevalence
100
90
80
70
60
50
40
30
20
10
0
Feb 94(26,39)
Sept 94(59,15)
Apr 95(42,12)
Date


Eimeria webbae
subadults
adults
Prevalence
100
90
80
70
60
50
40
30
20
10
0
Feb 94(26,39)
Sept 94(59,15)
Apr 95(42,12)
Date


Eimeria sigmodontis
pregnant
non-pregnant
Prevalence
100
90
80
70
60
50
40
30
20
10
0
Sept 94(25,13)
Oct 95(7,10)
Date


Eimeria webbae
pregnant
non-pregnant
Prevalence
100
90
80
70
60
50
40
30
20
10
0
Sept 94(25,13)
Oct 95(7,10)
Date

CHAPTER 3

ASSESSING THE POTENTIAL OF TERRESTRIAL ARTHROPOD COMMUNITIES AS BIOINDICATORS OF ECOTOXICOLOGICAL RISK, WITH SPECIAL REFERENCE TO ISOPODS

Introduction

Arthropods are among the most ubiquitous group of organisms in the world, serving vital functions in the environment as decomposers of organic materials and facilitators in the cycling of nutrients (Merchant and Crossley 1977). Because they are also an important source of food for numerous predatory vertebrates and invertebrates, they can also be important reservoirs for the accumulation of metals and other toxicants through the biosphere (Lindqvist and Block 1997). Consequently, arthropod communities have been the focus of considerable research interest as potential *in situ* indicators of ecotoxicity in terrestrial systems (Van Gestel and Van Straalen 1994).

The impact of environmental pollutants on the structure of terrestrial arthropod populations and communities has been widely documented. Reductions in densities of soil and surface-dwelling arthropods have been documented following exposure to copper (Hunter et al. 1983; Streit 1984), cadmium (Hunter et al. 1983; Van Straalen et al. 1987) lead (Siepel 1995) and pesticides (Perry 1997). A variety of sublethal responses have also been observed following exposure to contaminants, including increased growth rates and early onset of sexual maturation in collembolans and isopods exposed to heavy metals (Bengtsson et al. 1983; Donker et al. 1993). Increased reproductive allocation in isopods exposed to polycyclic aromatic hydrocarbons and metals has also been noted in several studies (Donker and Bogert 1991; Donker et al. 1993; Posthuma et al. 1993; Van Brummelen et al. 1996a). Contaminant-induced acclimation or adaptation (Frasier 1980: Donker et al. 1993) and endocrine disruption (Van Brummelen et al. 1996a) are among the suggested potential mechanisms responsible for these sublethal effects.

Despite the large volume of research on the sensitivity of arthropod species to pollutants, assessment of the effects of soil petrochemical contamination on arthropod communities, one of the most pervasive forms of environmental pollution in terrestrial ecosystems, has been largely ignored (Albers 1995). Environments that have been contaminated by the disposal of petrochemical wastes characteristically possess complex mixtures of heavy metals and organic hydrocarbons, which differ widely in their reactivity and bioavailabilty in the soil (Apitz 1996). Given the existing shortcomings, our primary objective was to examine the impact of soils contaminated with complex mixtures of petrochemical wastes on population and community structure of arthropods inhabiting tallgrass prairie ecosystems. Surface-dwelling macroarthropods and soil-dwelling microarthropods were surveyed in two reference and three contaminated environments on an abandoned oil refinery in southwest Oklahoma. Each of the contaminated environments contained unique mixtures of both metals and organic compounds that commonly accumulate in soils as a result of routine methods of refinery waste processing. Although both the reference and contaminated environments supported similar vegetative communities, we hypothesized that alterations in population densities and structure of arthropod communities would be observed between the populations residing on contaminated and reference environments.

Materials and Methods

Study Area

This study was conducted at the former Oklahoma Refining Company oil processing facility in Cyril, Oklahoma. The 73 ha site has been designated as a hazardous waste site on the Environmental Protection Agency's National Priorities List (Superfund) since it closed in 1984. A remedial investigation of this Superfund site (Stanley Engineering 1985) was used to aid in the selection of three contaminated sites for detailed study. We refer to these three sites as the storage pit, sludge trap, and land farm sites. The storage pit site was comprised of land immediately adjacent to several unlined pits used for the disposal and storage of asphalt processing wastes. The sludge trap site was comprised of land along a series of oil sludge sedimentation ponds that received process wastewater from the refinery and where trapped sludges were frequently deposited on the surface of earthen burms that surrounded the ponds. The soil farm site consisted of a 3.4 ha area where wastes from oil sludge traps, storage tanks, and other unidentified sources were tilled into the soil for biodegradation. We selected two reference study sites (designated reference sites 1 and 2) within 7 km from the refinery complex based on their ecological similarity in vegetative structure and composition to the contaminated sites. All five study sites were dominated by early successional, disturbance-adapted vegetation such as johnsongrass (*Sorghum halapense*), sumac (*Rhus* spp.), brome grasses (*Bromus* spp.), ragweed (*Ambrosia* spp.), and sagewort (*Artemisia* spp.). McMurry (1993) provided detailed descriptions of the vegetative composition and cover on each of these five study sites.

Previous surveys of the major soil contaminants on the contaminated sites documented the presence of heavy metals and various organic hydrocarbons from refining processes. Organic hydrocarbons were only documented on storage pit and

sludge trap sites. The primary hydrocarbons found were toluene, xylene, and pyrene (Stanley Engineering Inc. 1985; USEPA 1985). Elevated levels of several heavy metals were also documented, with observed levels ranging from 24 to 2700 ppm for chromium and 14 to 304 ppm for lead on all three of the contaminated sites. Arsenic was detected in soil samples from the storage pit and sludge trap sites, with averages of 104 ppm and 3 ppm, respectively. Elevated levels of aluminum, barium, zinc, and mercury were observed only in soil samples from the storage pit site, with levels ranging from 19 to 53,800 ppm (Stanley Engineering Inc. 1985; USEPA 1985).

Data Collection

We sampled both macroarthropod and microarthropod communities during late Summer and Fall 1995. Sampling of the macroarthropod communities was replicated 3 times from late August to early October. A 3 by 4 grid of pit-fall traps was established on each site, with a 3.2m distance between each trap station. Traps were filled up to ca. 5 cm with dilute ethanol and remained open for 48 hours during each sampling period. All arthropods were removed at the end of the sampling period and placed in plastic jars containing 70% ethanol until identification and enumeration could be undertaken. Insects and arachnids were identified to Family and categorized into general taxonomic groups for relative density comparisons between the contaminated and reference sites. Other macroarthropod groups were identified to Class (Isopoda, Symphyla, Chilopoda, and Diplopoda).

Sampling of the microarthropod communities was replicated 5 times from late August to early October 1995. During each sampling period we removed four soil cores

from each study site. In all cases the soil core samples were collected between 7:00 am and 9:30 am to minimize the effects of diurnal arthropod movements through the soil column (McBrayer et al. 1977; Seastedt and Crossley 1981). Cylindrical cores of the top 5 cm of soil were extracted with 3.3-cm. diameter pieces of plastic pipe that were sharpened at one end to facilitate movement through the soil. Soil cores were immediately wrapped in aluminum foil and stored on ice to reduce arthropod movements until they could be returned to the laboratory within 2-4 hours (Seastedt and Crossley 1981). Once returned to the laboratory, the samples were placed in a Tullgren-type extractor for 7 days (Merchant and Crossley 1970). Microarthropods were collected and stored in 70% ethanol until they could be identified. Mites (Acarina) were identified to Suborder, and other microarthropods were identified to Order.

Data Analysis

Indices of density for arthropod populations were analyzed statistically using a completely randomized design with repeated measures. The densities of common groups of microarthropods (individuals/m^2) and relative densities (mean number of individuals/ trap) of macroarthropods were analyzed using PROC MIXED (SAS 1997), with sources of variation in the model consisting of study site (5 sites), treatment (reference, contaminated), site within treatment (error term for treatment), sampling period, treatment by sampling period interaction, and the residual. A symmetric compound model was used to model the covariance structure of the repeated measures. If the treatment by sampling date interaction was significant, simple effects of treatment were analyzed using the SLICE option for the LSMEANS statement. Satterthwait's approximation was used

for the calculation of degrees of freedom of the pooled error. If the sampling date by treatment interactions were not significant, the main effects were analyzed using LSMEANS with the DIFF option. Densities of major microarthropod and macroarthropod groups were transformed by log (x+1) prior to analysis and statistical significance for all tests was set *a priori* at $P \leq 0.05$.

Diversity of macroarthropod and microarthropod communities was measured by the complement of Simpson's diversity (Krebs 1989), transformed with an arcsine function and analyzed with analysis of variance for repeated measures as described above for density comparisons. Comparisons of similarity of species composition and abundances in communities were measured using Horn's index (Horn 1966) with the results presented as a single-linkage cluster diagram as per Krebs (1989).

Results

Macroarthropod Communities

Relative densities of total macroarthropod communities were greater ($P \leq 0.005$) on the contaminated than reference sites and also varied significantly ($P \leq 0.0001$) across the three sampling periods (Tables 1 and 2). A significant treatment by sampling period interaction ($P \leq 0.0001$) was indicated for the total macroarthropod relative community densities, which decreased 62 to 71% on contaminated study sites by the 24 October survey compared to the two earlier surveys, while relative densities on reference sites remained stable (Table 1).

Although total relative densities of insects were not influenced ($P > 0.05$) by treatment or sampling period, there was a significant ($P \leq 0.05$) treatment by sampling period interaction indicated because relative density was over 30% lower on reference sites on 24 October compared to contaminated areas, but were similar between treatments during other sampling periods (Table 1). Relative densities of hymenopterans were 50 to 100 % greater ($P \leq 0.01$) on contaminated than reference sites in both October sampling periods. Relative densities of coleopterans (beetles) were not different ($P > 0.05$) between sampling periods and treatments (Table 1). Although the main effects of sampling period and treatment did not ($P > 0.05$) affect relative densities of arachnids, there was a significant treatment by sampling period interaction ($P \leq 0.0005$). Relative densities of arachnids were four-fold greater on contaminated sites compared to reference sites on 24 October. Relative densities of the Araneae showed a significant ($P \leq 0.05$) treatment by sampling period interaction due to lower densities on the contaminated sites on 10 October and on the reference sites on 24 October.

Overall relative densities of isopods were 180-fold greater ($P < 0.005$, Fig. 1) on the contaminated sites than on the reference sites. A significant treatment by sampling period interaction ($P \leq 0.0001$) was also indicated as the relative densities of isopods on the contaminated sites declined by over 80% on 24 October compared to 29 August and 10 October. Relative densities of isopods remained consistently low (mean of less than one isopod per trap) on reference sites throughout all sampling periods.

Comparisons of similarity and diversity indices of macroarthropod communities indicated that soil pollution had significant effects on overall community structure. Diversity of macroarthropod communities was observed to be influenced ($P \leq 0.05$) by

soil contamination. Diversities were 35-45% greater on the reference sites on 29 August and 10 October, but were similar in the 24 October sampling period (Fig. 4). Comparisons of relative abundances and species composition of macroarthropod communities on contaminated and reference sites, using Horn's index of similarity (Horn 1966), revealed that the three contaminated sites clustered together for all three of the sampling periods, with discrimination between the reference and contaminated sites most pronounced on 29 August and 10 October (Fig. 2). During the 29 August and 10 October sampling periods the contaminated sites all clustered together at > 90% similarity, while similarity between the reference and contaminated sites was below 50%. The results of the 24 October sampling period were less clear- the three contaminated sites clustered together at 83% similarity, but reference site 2 clustered out with the contaminated sites rather than with the other reference site.

Microarthropod Communities

Comparisons of the relative densities for each of the eight microarthropod groups found showed that soil contaminants had little or no measurable impact ($P > 0.05$) on the community structure of these organisms (Table 3). Total insect densities were lower in September collections compared to both the August and October sampling periods ($P < 0.01$; Table 4). Collembolan densities were also influenced sampling period, decreasing more than 50% over the course of the study (Table 3).

Comparisons of the similarity of relative abundances and species composition revealed poor discrimination between the reference and contaminated sites, where reference site 1 clustered with the land farm site (Fig. 3). Diversity indices among the

communities on the reference (0.24 ± 0.04) and contaminated (0.30 ± 0.05) sites were similar as well ($P > 0.05$).

Discussion

The results of this study suggested that macroarthropod communities were more sensitive to the complex mixtures of contaminants associated with oil refinery wastes than microarthropod communities. This was an unexpected observation, especially with regards to the apparent insensitivity of oribatid mites and collembolans to the contaminants in the soil. Because soil arthropods such as these reside in direct contact with the soil, sensitivity to soil contaminants through direct contact and ingestion of soil and soil water would appear to be high (Sheppard and Evenden 1994; Lebrun and Van Straalen 1995). This contention is supported by several studies that have demonstrated the sensitivity of oribatid mites and collembolans to various forms of soil pollution. For example, Stamou and Argyropoulou (1995) observed declines in the densities of oribatid mites inhabiting metal-contaminated urban areas, primarily due to the observation that some species of mites were highly sensitive compared to others. Although overall densities of oribatids declined, these authors noted that the more common species of mites found in their study were actually greater in density in the contaminated soils than on their reference sites. In another study, Steiner (1995) documented a decline in diversity of collembolan communities from 15 species to 2 in areas that were chronically polluted with automobile exhaust. However, Perry et al. (1997) observed decreases in collembolan densities in leaf litter following the application of the gypsy moth

(*Lymantria dispar* L.) suppressant diflubenzuron in forested habitats, but did not observe decreases in oribatid communities. It is also possible that adaptation to contamination has occurred on our study sites, which has been demonstrated in some collembola species (Van Straalen et al. 1987; Posthuma et al. 1992). Given the complex and highly variable responses of individual oribatid and collembolan species to soil contaminants, it is certainly possible that individual populations may have been altered in our study communities without a change in total densities of oribatids and collembolans.

We observed a wide range of responses of macroarthropod groups to soil contamination in our study. Relative densities of two groups, isopoda and hymenoptera, were elevated on contaminated compared to reference sites. Relative densities of total insects, total arachnids, and Araneae demonstrated significant interactions of treatment and sampling period, indicating that soil contaminants may have altered seasonal cycles in abundances of these communities. Relative densities of coleopeterans were similar between treatments, suggesting that this group were not strongly affected by soil contaminants.

Hymenopterans have been shown to possess the ability to tolerate high levels of soil contamination. Koponen and Niemela (1995) did not observe density differences in ants along a pollution gradient for both a fertilizer and smelter plant. They also found permanent hymenopteran populations living in the most heavily contaminated areas of the two industrial plants.

The most remarkable difference that we observed was undoubtedly the enormous differences in the densities of terrestrial isopods between contaminated and reference areas. Isopods were the dominant macroarthropod group on all contaminated sites,

frequently comprising > 90% of the total individuals sampled. Although isopods occurred on the reference sites, there were never more than two individuals in a sample. These differences in relative densities could have been caused by exposure to certain toxicants on the site, some of which have been demonstrated to increase reproductive allocation and output in certain isopod species. For example, Van Brummelen et al. (1996a) observed a significant stimulation of reproduction in laboratory populations of *Oniscus asellus* following exposure to fluoranthene, phenanthrene, benz[a]anthracene, and benzo[a]pyrene, which isopods do accumulate in the environment (Van Brummelen et al. 1996b). Donker et al. (1993) observed early onset of sexual maturation and increased reproductive allocation in populations of the isopod *Porcellio scaber* inhabiting areas near a zinc smelter and a lead mine. *Porcellio scaber* populations have also been shown to adapt to high levels of cadmium, which was also observed to stimulate growth in populations residing in close proximity to a zinc smelter (Donker and Bogert 1991). In contrast, Hunter et al. (1987) observed decreased population densities of isopods in grassland areas contaminated with copper and cadmium. It seems likely that the high densities of isopods that we observed on the contaminated sites were due to increased recruitment rates, although indirect effects of contamination (such as direct effects of contamination on predators of isopods) could not be ruled out as well. The decrease in isopod densities that we observed on contaminated sites in the October collections probably reflected normal reductions in reproduction due to changes in photoperiod and temperature (Warburg and Weinstein 1995).

As a result of the dominance of isopods on the contaminated sites, macroarthropod communities residing on these sites were more similar to one another

than to those on the reference sites. This similarity was less distinguishable in the last survey, when isopods declined, but all contaminated sites still clustered together before the reference sites.

Cumulatively these observations support our original hypothesis that petrochemical contamination can alter macroarthropod community structure. However, the impact of these complex mixtures of soil contaminants on microarthropod communities was not discernible in our study. Our results indicate that macroarthropod communities can be a useful addition to ecotoxicity assessments of terrestrial ecosystems. In particular, isopod populations appear to be most useful as bioindicators of toxicants in soils that have been contaminated with petrochemical waste products generated in the refining of petroleum.

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Table 1. Relative densities (mean number of individuals/trap) of major macroarthropod groups collected from three contaminated and two reference sites on the Oklahoma Refining Co. Superfund waste site, Caddo County, Oklahoma, August to October 1995.

	Total macroarthropods		Total insects		Hymenoptera		Coleoptera		Total arachnids		Araneae	
Site/sampling period	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Reference sites												
29 August 1995	17.5	2.4	15.8	2.3	10.4	2.2	1.6	0.3	1.3	0.2	1.0	0.2
10 October 1995	15.6	1.6	14.4	1.7	4.8	0.7	2.2	0.5	1.0	0.2	0.7	0.2
24 October 1995	11.0	1.6	10.0	1.5	5.5	1.4	1.2	0.2	0.6	0.2	0.4	0.1
Contaminated sites												
29 August 1995	91.2	9.2	15.9	1.9	10.9	1.8	2.7	0.4	1.8	0.3	1.4	0.3
10 October 1995	69.4	4.5	17.6	1.5	9.6	1.0	2.7	0.4	0.9	0.2	0.5	0.1
24 October 1995	26.2	1.9	16.3	1.3	8.4	0.9	3.3	0.4	2.5	0.3	1.6	0.3

Table 2. *F* and *P* values generated by analysis of variance for repeated measures for main effects of treatment, date and interactions between relative densities of selected macroarthropod groups from two reference and three contaminated sites on the Oklahoma Refining Co. Superfund waste site, Caddo County, Oklahoma, August to October 1995.

		Total macroarthropods		Total insects		Hymenoptera		Coleoptera		Total arachnids		Araneae	
	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Treatment	1	94.05	0.0023	1.97	0.2550	11.24	0.0100	6.19	0.0882	1.81	0.2711	1.51	0.3063
Sampling period	2	46.88	0.0001	1.85	0.1599	2.74	0.0675	0.04	0.9650	2.80	0.0639	3.19	0.0437
Treatment x Sampling period	2	10.05	0.0001	3.15	0.0452	1.86	0.1584	2.06	0.1321	8.37	0.0003	5.55	0.0046

Table 3. Mean population densities (individuals × $10^3/m^2$) of major microarthropod groups collected from three contaminated and two reference areas of the Oklahoma Refining Co. Superfund waste site, Caddo County, Oklahoma. Values represent pooled data across surveys from August to October 1995.

	Total microarthropods	Total insects	Order collembola	Total arachnids	Order Acarina	Suborder actinedida	Suborder gamasida	Suborder oribatida
Reference(x ± SE)	12.3 ± 1.7	5.2 ± 1.1	1.9 ± 0.6	6.9 ± 0.9	6.6 ±	1.3 ± 0.4	0.5 ± 0.1	4.8 ± 0.6
Contaminated (x ± SE)	13.1 ± 1.3	3.3 ± 0.6	2.1 ± 0.5	9.4 ± 1.1	8.9 ±	2.1 ± 0.4	0.7 ± 0.2	6.1 ± 0.8

Table 4: F and P values generated by ANOVA for repeated measures for main effects of treatment, sampling date, and interactions for major microarthropod groups collected from three contaminated and two reference areas of the Oklahoma Refining Co. Superfund waste site, Caddo County, Oklahoma. Values represent pooled data across surveys from Fall 1995.

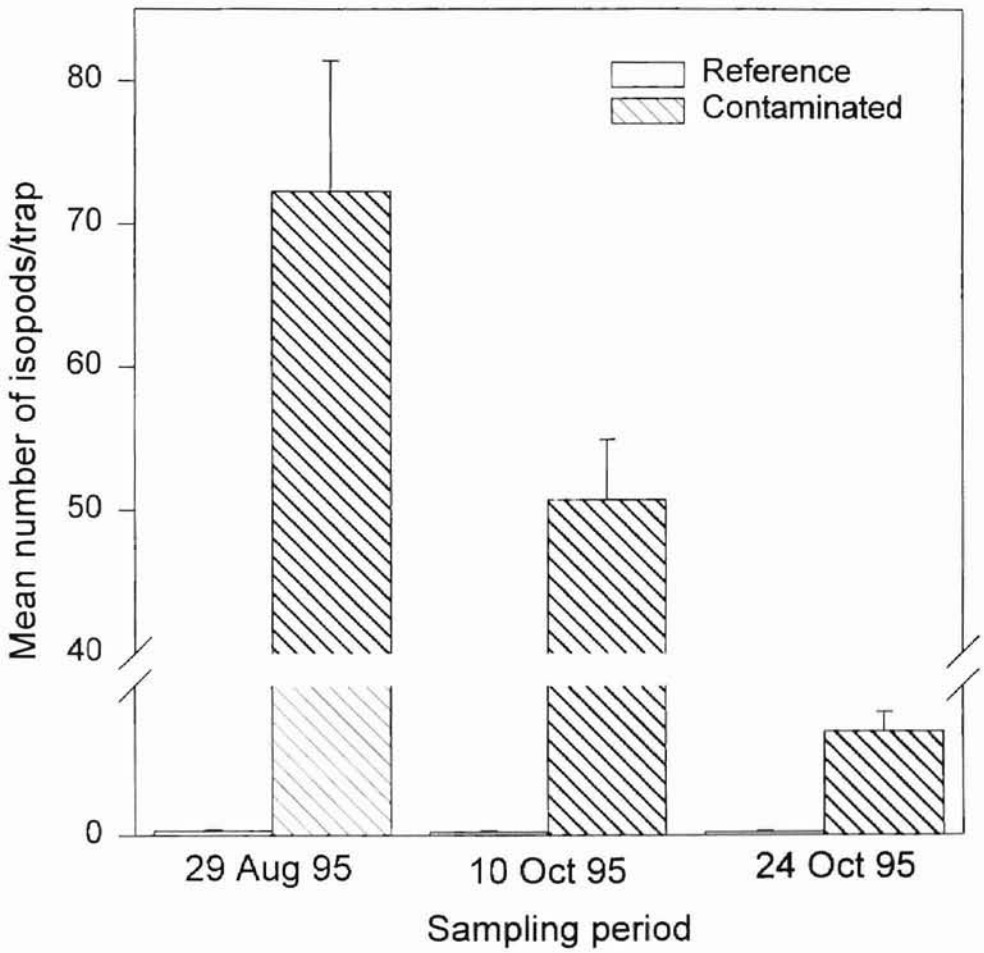
		Total microarthropods	Total insects	Order collembola	Total arachnids	Order Acarina	Suborder actinedida	Suborder gamasida	Suborder oribatida
Treatment (1 df)									
	F	0.89	0.30	0.14	1.70	0.67	1.51	0.39	0.07
	P	0.3472	0.6201	0.7108	0.1954	0.4139	0.0306	0.5787	0.7943
Sampling Period (4 df)	F	0.97	3.89	4.89	1.28	1.67	0.81	0.97	1.73
	P	0.4272	0.0059	0.0013	0.2825	0.1639	0.5209	0.4278	0.1497
Treatment x Sampling Period (4 df)	F	1.76	0.80	0.36	1.33	1.27	1.38	1.42	0.41
	P	0.1430	0.5257	0.8367	0.2645	0.2875	0.2492	0.2343	0.8000

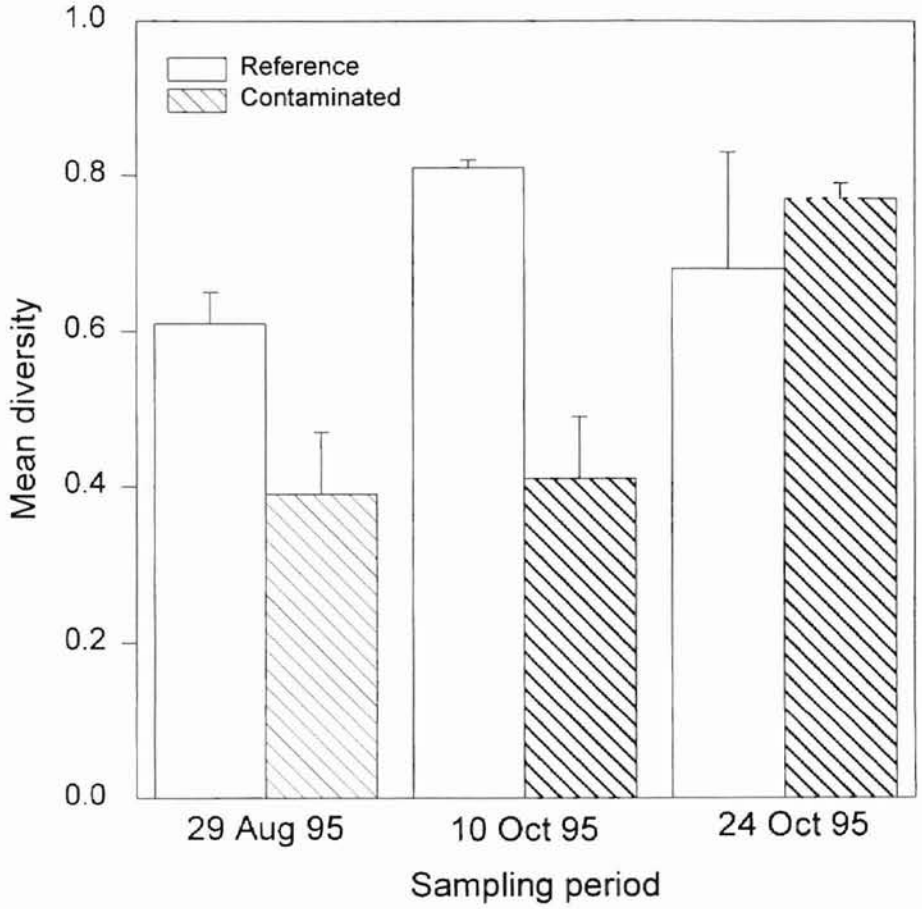
Figure 1: Relative densities (average number of individuals per trap) of terrestrial isopods recovered from contaminated and reference sites on the Oklahoma Refining Company superfund site, Caddo County, Oklahoma, August to October 1995.

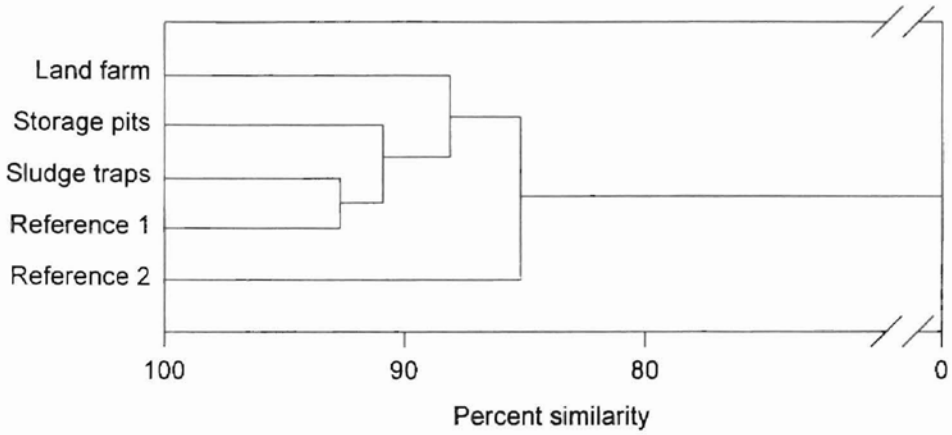
Figure 2: Mean diversity of macroarthropod communities residing on contaminated and reference sites on the Oklahoma Refining Company superfund site, Caddo County, Oklahoma, August to October 1995

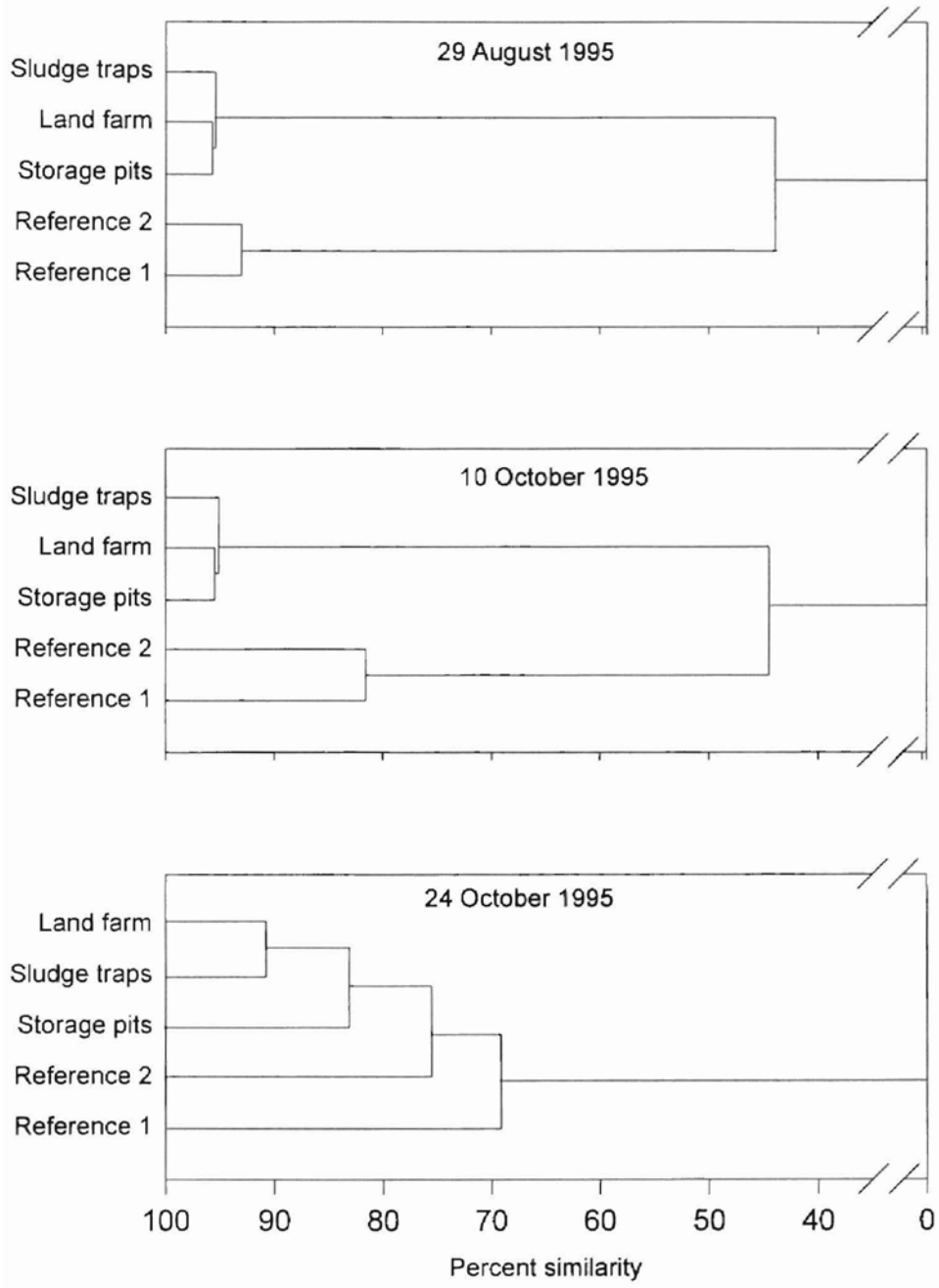
Figure 3: Single-linkage cluster diagrams depicting the similarity (Horn 1966) of microarthropod communities surveyed on three contaminated and two reference sites on the Oklahoma Refining Company superfund waste site, Caddo County, Oklahoma. Comparisons of similarity were performed on data pooled from collections from August to October 1995.

Figure 4: Single-linkage cluster diagrams depicting the similarity (Horn 1966) of macroarthropod communities surveyed on three contaminated and two reference sites on the Oklahoma Refining Company superfund waste site, Caddo County, Oklahoma, August to October 1995.


Reference
Contaminated
Mean number of isopods/trap
80
70
60
50
40
0
29 Aug 95
10 Oct 95
24 Oct 95
Sampling period


1.0
0.8
0.6
0.4
0.2
0.0
Mean diversity
Reference
Contaminated
29 Aug 95
10 Oct 95
24 Oct 95
Sampling period


Land farm
Storage pits
Sludge traps
Reference 1
Reference 2
100
90
80
0
Percent similarity


29 August 1995
Sludge traps
Land farm
Storage pits
Reference 2
Reference 1
10 October 1995
Sludge traps
Land farm
Storage pits
Reference 2
Reference 1
24 October 1995
Land farm
Sludge traps
Storage pits
Reference 2
Reference 1
100
90
80
70
60
50
40
0
Percent similarity

VITA 2

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