

FINAL TECHNICAL REPORT

AASERT Project Control No. 93-NL-074

31 August 1997

**AN INEXPENSIVE MODEL SYSTEM FOR IN SITU EVALUATIONS OF ECOLOGICAL
RISK IN PETROCHEMICAL-CONTAMINATED TERRESTRIAL ENVIRONMENTS**

Robert L. Lochmiller, Ph.D.

Principal Investigator

**Environmental Toxicology Program
Department of Zoology
Oklahoma State University
Stillwater, OK 74078**

DTIC QUALITY INSPECTED 2

19980210 063

An Inexpensive Model System for in situ Evaluations of Ecological Risk in Petrochemical-Contaminated Terrestrial Environments

EXECUTIVE SUMMARY

The overall objective of this research was further elucidate the mechanisms by which petrochemical contaminants influence terrestrial ecosystems and to develop reliable in situ biomonitoring tools for assessing risks to mammalian systems and ecosystems. Specifically, we approached this objective by examining the feasibility of using internal parasite communities harbored by resident small mammals as an in situ assay system for evaluating impacts of petrochemical contamination on complex ecological processes in terrestrial environments. Host parasite community ecology represents a culmination of a myriad complex ecological processes, including interactions among hosts, intermediate hosts, and parasites, host immunity, direct toxicity to parasites, and other processes.

Overall effects of petrochemical soil contamination on the structure (species composition and abundances) of the parasitic communities of cotton rats were evident in the single-linkage cluster analysis of similarity indices. 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. Higher relative densities of surface-dwelling macroarthropod groups were observed on the contaminated sites. The most notable difference was the terrestrial isopods, which had densities 180 times higher on contaminated than reference sites. Significant differences were also noted in macroarthropod diversity, and the results of similarity comparisons of macroarthropod communities residing on the contaminated and reference sites indicated community abundances and composition on the contaminated sites were more similar to one another than to the communities residing on the reference sites. The results of our survey suggest that helminth parasite communities of cotton rats and macroarthropod communities in the habitat could be valuable ecosystem-level bioindicators for evaluating the effects of soil contamination on terrestrial systems.

REPORT PERIOD

1 September 1993 to 31 August 1997

PRINCIPAL INVESTIGATOR

Dr. Robert L. Lochmiller

OTHER PROJECT PERSONNEL SUPPORTED

□

Listed below is a list of personnel that have been associated with the USAF-supported project during the life of the project; some have been supported directly while others have been associated indirectly with project as collaborators or volunteers:

Dr. Robert L. Lochmiller (PI: Professor, OSU)
Dr. William Warde (Statistical consultant, Professor, OSU)
Dan Rafferty (M.S. candidate: 1 August 1995 - present)
Lee Jones (Technician 100% effort: 1 April 1995 - present)
Brian Faulkner (M.S. student: assisted with census, summer 1995)
Tim Propst (M.S. student: assisted with summer census)
Mike G. Sams (M.S. student: assisted with summer census)
Tim Schetter (M.S. student: assisted with summer census)

RESEARCH OBJECTIVES**Objective I**

To evaluate the effects and sensitivity of various population and community measures of organization for parasitic communities harbored by resident small-mammalian hosts inhabiting terrestrial environments contaminated with complex mixtures of petrochemicals.

a) Monitor seasonal (winter, summer) changes in the prevalence, over-dispersion, abundance, and intensity of infection for each species of parasite infecting resident small mammals collected from contaminated and uncontaminated reference sites.

b) Describe seasonal changes in organization (diversity, species richness) and compare structural similarities of parasite communities among all study sites.

Objective II

To examine how abundance and diversity of host communities and availability of intermediate parasite hosts influence the seasonal diversity, structure, and composition of parasite communities on contaminated environments.

There has been no change in the research objectives listed in the original proposal.

RESEARCH APPROACH

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 sites 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 contaminated sites, and the primary contaminants were toluene, xylenes, pyrene, anthracene, naphthalene, 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, with 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-carmin stain mounts for cestodes; total recovery of all helminth species was attempted.

We sampled both macroarthropod and microarthropod communities during late Summer and Fall 1995. Sampling of the macroarthropod communities was replicated three times and we a 3x4 grid of pit-fall traps on each site. All arthropods were removed at the end of the sampling were identified and enumeration to family, and categorized into general taxonomic groups for relative density comparisons between the contaminated and reference sites. Other macroarthropod groups were identified into Classes (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. 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) for Tullgren extraction (Merchant and Crossley 1970) for 7 days. Microarthropods were collected and mites (Acarina) were identified to Suborder, and other microarthropods were identified to Order.

Data Analysis

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 $P \leq 0.05$.

RESEARCH OVERVIEW

Helminth Fauna and Prevalence

All but four of the 340 cotton rats which we collected were infected with at least one species of helminth (Table 1). Three species of cestodes (*Raillietina sigmodontis*, *Schizotaenia sigmodontis*, and *Hymenolepis diminuta*) and four species of nematodes (*Protospirura muris*, *Longistriata adunca*, *Syphacia sigmodontis*, and *Strongyloides sigmodontis*) were recovered. *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 diminuta* 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 1). *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. 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 ($P < 0.05$). 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. 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. Abundances of infection with *Hymenolepis diminuta* 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 diminuta* in cotton rats from the reference sites in October 1995.

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 1). 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. 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.

Helminth Community

Mean species richness of gastrointestinal parasite communities within host populations was significantly influenced by treatment ($P \leq 0.05$; Table 2). Host animals on the reference sites consistently supported a greater average number of helminth species than those from contaminated sites in all seasonal collections. Mean species richness also differed across seasons ($P < 0.05$), being significantly higher in September 1994 than in April and October 1995. 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.

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.

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 some of this difference is the result of impacts (positive or negative) from soil contamination on the availability of intermediate hosts (see below in Arthropod section), which could play a profound role in the regulation of these helminth species in their hosts.

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.

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.

Arthropod Community

Relative densities of the 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 collection dates (Table 3). A significant treatment by sampling date interaction ($P \leq 0.0001$) was indicated because total macroarthropod relative densities decreased 62 to 71% on contaminated study sites by the 24 October survey compared to the two earlier surveys, while the relative densities on the reference sites remained stable. Relative densities of hymenopterans were 50 to 100 % greater ($P \leq 0.01$) on contaminated than reference sites in the October surveys. Relative densities of coleopterans (beetles) were not different ($P > 0.05$) between sampling dates or treatments remaining fairly constant across all surveys (Table 3). Although the main effects of sampling date and treatment did not ($P > 0.05$) affect relative densities of total arachnids, there was a significant treatment by sampling date 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 Order Araneae showed a significant ($P \leq 0.05$) treatment by sampling date interaction due to lower densities on the contaminated sites on 10 October and on the reference sites on 24 October. In both of these surveys the densities of Order Araneae were 50 to 60% lower on these sites than the other surveys of the reference and contaminated sites.

Overall isopod relative densities were 180-fold greater ($P < 0.005$, Fig. 4) on the contaminated sites than on the reference sites. A significant treatment by sampling date interaction ($P \leq 0.0001$) was also indicated as the relative densities of isopods on the contaminated sites decreased over 80% on 24 October compared to the 29 August and 10 October surveys. Relative densities of isopods remained consistently low (an average of less than one isopod per trap) on the reference sites throughout all survey periods.

Comparisons of the densities of eight microarthropod groups showed that soil contaminants had little or no measurable impact on the densities of populations in this study (Table 4)

Comparisons similarity and diversity of macroarthropod communities indicated that soil pollution had significant effects on community structure. Comparisons of relative abundances and species composition of macroarthropod communities on contaminated and reference sites revealed that the contaminated sites clustered together for all three of the collections, with the discrimination between the reference and contaminated sites most pronounced on 29 August and 10 October (Fig. 5). In the 29 August and 10 October surveys the contaminated sites all clustered together at $> 90\%$ similarity, while similarity between the reference and contaminated sites was below 50%.

Our results suggest that macroarthropod communities are considerably more sensitive to the complex mixtures of contaminants associated with oil refinery waste than microarthropod communities. 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 group on the contaminated sites, frequently comprising $> 90\%$ of the total arthropods surveyed. Although isopods occurred on the reference sites, there were never more than two

individuals in a sample. This enormous difference 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* which inhabited areas near a zinc smelter and a lead mine compared to populations in uncontaminated areas. *Porcellio scaber* populations have also been shown to adapt to high levels of cadmium, which was also observed to stimulate growth in isopod populations which resided in close proximity to a zinc smelter (Donker and Bogert 1991). In contrast, Hunter et al. (1987) observed lower population densities of isopod populations in grassland areas contaminated with copper and cadmium. It seems likely that the high densities of isopods we observed on the contaminated sites in our study 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.

RESULTING CREATIVE ACCOMPLISHMENTS ON THE PROJECT

Publications

- Faulkner, B.F., and R.L. Lochmiller. 1997. New geographic records of coccidia (apicomplexa) from the cotton rat in Oklahoma. *Proc. Okla. Acad. Sci.* (in press)
-
- Lochmiller, R. L. 1996. Immunocompetence and animal population regulation. *Oikos* (in press).
- McBee, K., and R. L. Lochmiller. 1996. Wildlife toxicology in biomonitoring and bioremediation: Implications for human health. In *A biological approach to environmental remediation: Ecotoxicity and human health* (A. D. Bloom and F. J. de Serres, eds.). CRC Press, pp. 163- 181.
- Robel, G. L., R. L. Lochmiller, S. T. McMurry, and C. W. Qualls. 1996. Environmental, age, and gender effects on cotton rat (*Sigmodon hispidus*) hematology. *J. Wildl. Dis.* 32(2):390-394.
- McMurry, S. T., R. L. Lochmiller, J. F. Boggs, D. M. Leslie, and D. M. Engle. 1996. Demography and condition of white-footed mouse (*Peromyscus leucopus*) populations in late and early successional habitats. *J. Mammal.* 77(2):335-345.
- Lochmiller, R. L. 1995. Testing the immunocompetence handicap theory. *Trends Ecol. Evol.* 10(9):372-373.
- Lochmiller, R. L. 1995. Testing the immunocompetence handicap theory. *Trends Ecol. Evol.* 10(9):372-373.
- Propst, T. L., R. L. Lochmiller, and C. W. Qualls. 1995. Using mesocosms to assess immunotoxicity risks in terrestrial environments contaminated with petrochemicals. In: *The Effects of Oil on Wildlife: Response, Research &*

- Contingency Planning (Frink, L., K. Ball-Weir, and C. Smith, eds.), Tri-State Bird Rescue & Research, Inc., Newark, DE. pp. 160-166.
- Davis, R., and R. L. Lochmiller. 1995. Quantitative and qualitative numerical alterations in splenocyte subpopulations of the cotton rat (*Sigmodon hispidus*) across seasons. Biol. Rhythm Res. 26(1):20-31.
- Davis, R., R. L. Lochmiller, and W. Warde. 1995. Splenocyte subpopulations of weanling cotton rats (*Sigmodon hispidus*) are influenced by moderate protein intake. *J. Mammal.* 76(3):912-924.
- Dabbert, C. B., R. L. Lochmiller, J. R. Zhing, and C. W. Qualls, Jr.. 1994. High in vivo endotoxin responsiveness of macrophages from an endotoxin-resistant wild rodent species, *Sigmodon hispidus*. *Dev. Comp. Immunol.* 18:147-153.
- Lochmiller, R. L., M. R. Vestey, and S. T. McMurry. 1994. Temporal variation in humoral and cell-mediated immune response in a *Sigmodon hispidus* population. Ecology 75:236-245.
- Propst, T. L., R. L. Lochmiller, and C. W. Qualls. 1995. Using mesocosms to assess immunotoxicity risks in terrestrial environments contaminated with petrochemicals. In: *The Effects of Oil on Wildlife: Response, Research & Contingency Planning* (Frink, L., K. Ball-Weir, and C. Smith, eds.), Tri-State Bird Rescue & Research, Inc., Newark, DE. pp. 160-166.
- McMurry, S. T., R. L. Lochmiller, S. A. M. Chandra, and C. W. Qualls. 1995. Sensitivity of selected immunological, hematological, and reproductive parameters in the cotton rat (*Sigmodon hispidus*) to subchronic lead exposure. *J. Wildl. Dis.* 31(2):193-204.
- McMurry, S. T., R. L. Lochmiller, M. R. Vestey, and C. W. Qualls, Jr. 1994. Immunological responses of weanling cotton rats (*Sigmodon hispidus*) to acute benzene and cyclophosphamide exposure. *Bull. Environ. Contam. Toxicol.* 52:155-162.
- McMurry, S. T., R. L. Lochmiller, M. R. Vestey, and C. W. Qualls, Jr. 1994. Cellular immune responses of nutritionally stressed juvenile cotton rats (*Sigmodon hispidus*) during acute benzene exposure. *Arch. Environ. Contam. Toxicol.* 27:14-19.
- Paranjpe, M. G., A. M. Chandra, C. W. Qualls, S. T. McMurry, M. D. Rohrer, M. M. Whaley, R. L. Lochmiller, and K. McBee. 1994. Fluorosis in a wild cotton rat (*Sigmodon hispidus*) population inhabiting a petrochemical waste site. Toxicol. Pathol. 22(6):569-578.

Presentations

- Faulkner, B.F., and R.L. Lochmiller. 1996. Utilizing the internal parasite communities of cotton rats to assess environmental risk. Soc. Environ. Toxicol. Contam., Wash. D.C., Nov. 11-17, 1996.
- Faulkner, B.F., and R.L. Lochmiller. 1995. The influence of petrochemical contamination on the cotton rat parasite *Protospiura muris*. Oklahoma Acad. Sci., Norman, Okla, Nov. 8, 1995.

- Boyd, C., R. Lochmiller, and C. Dabbert. 1994. Interrelationships between immunocompetence and population dynamics in small mammals. Okla. Acad. Sci. Annual Meeting, Norman, OK, 12 Nov.
- Chandra, S., C.W. Qualls, G. Reddy. 1994. Trinitrobenzene induced encephalopathy in male F-344 rats. Am. Coll. Toxicol., 15th Annual. Meet., Williamsburg, VA, Oct 1994.
- Faulkner, B., and R. Lochmiller. 1994. Factors influencing the population of the cotton rat parasite *Protospiura muris* on a superfund waste site. Oklah. Acad. Sci. Ann. Mett., Norman, OK, 12 Nov.
- McBee, K., C. Butchko, R. Lochmiller, T. Propst, and S. Burks. 1995. Genetic effects of petrochemical pollutants on cotton rats (*Sigmodon hispidus*) in mesocosms at a Superfund Waste Site. Am. Soc. Mammal.; Univ. Vermont, Burlington, VT, 20-24 June.
- Qualls, C.W., G. Reddy, and S. Chandra. 1995. Hematological and pathological effects of 1,3,5-trinitrobenzene in F-344 rats. Intern. Congr. Toxicol.-VII, Seattle, WA, July 1995.
- Reddy, G., C.W. Qualls, A.E. Gunnarson. 1994. Biochemical effects of 1,3,5-trinitrobenzene in F-344 rats. Intern. Congr. Biochem. Molecul. Biol., New Deli, India, Sept. 1994.
- Reddy, G., C.W. Qualls, A.E. Gunnarson. 1994. Effect of 1,3,5-trinitrobenzene on drug metabolizing enzymes in F-344 rats. Soc. Toxicol., March, Toxicologist 14:194.
- Zhigulina, E., and R. Lochmiller. 1994. Environmental influences on weight dynamics of the thymus gland in *Sigmodon hispidus*. Okla. Acad. Sci. Annual Meeting, Norman, OK, 12 Nov.

LITERATURE CITED

- Donker, M.H., and C.G. Bogert. 1991. Adaptation to cadmium in three populations of the isopod *porcellio scaber*. *Comp. Biochem. Physiol.* **100C**: 143-146.
- Donker, M.H., C. Zonneveld, and N.M. Van Straalen. 1993. Early reproduction and increased reproductive allocation in metal-adapted populations of the terrestrial isopod *Porcello scaber*. *Oecologia.* **96**: 316-323.
- Horn, H. S. 1966. Measurement of "overlap" in comparative ecological studies. *Am. Nat.* **100**: 419-424.
- Hunter, B.A., M. S. Johnson, and D.J. Thompson. 1983. Ecotoxicology of copper and cadmium in a contaminated grassland system II. Invertebrates. *J. Appl. Ecol.* **24**: 587-599.
- Krebs, C. J. 1989. *Ecological Methodology*. Harper Collins Publishers, New York, NY.
- Melvin, D. M. and A. C. Chandler. 1950. New helminths from the cotton rat, *Sigmodon hispidus*, including a new species, *Strongyloides sigmodontis*. *Journal of Parasitology* **36**: 505-510.
- Merchant, V.A. and D.A. Crossley. 1970. An inexpensive high efficiency tullgren extractor for soil microarthropods. *J. GA. Entomol. Soc.* **5**:83-87.
- SAS Institute, Inc. 1996. *SAS/STAT® Software: Changes and Enhancements Through Release 6.11*. SAS Institute, Inc. Cary, NC.
- Seastedt, T.R., and D.A. Crossley, Jr. 1981. Microarthropod response following cable logging and clear-cutting in the southern Appalachians. *Ecology* **62**: 126-135.
- Stanley Engineering, Inc. 1985. Oklahoma Refining Company environmental and hydrological assessment. Oklahoma City, Okla.
- USEPA, 1985. RCRA facility preliminary review report, Oklahoma Refining Company, Cyril, Oklahoma, Project No. W68439.
- Van Brummelen, T.C., C.A.M Van Gestel, and R.A. Verweij. 1996a. Long term toxicity of five polycyclic aromatic hydrocarbons for the terrestrial isopods *Oniscus asellus* and *Porcellio scaber*. *Environ. Toxicol. Chem.* **15**:1199-1210.
- Van Brummelen, T.C., R.A. Verweijz, S.A. Wedzinga, and C.A.M. Van Gestel. 1996b. Polycyclic aromatic hydrocarbons in earthworms and isopods from contaminated forest soils. *Chemosphere* **32**:315-341.

Table 1. Means and standard errors of abundances of gastrointestinal helminth infections in cotton rats from the reference and contaminated sites on the Oklahoma Refining Company site, September 1993 to October 1995.

Helminth species	Treatment	September 1993		February 1994		September 1994		April 1995		October 1995	
		Mean	S E	Mean	S E	Mean	S E	Mean	S E	Mean	S E
<i>Raillietina sigmodontis</i>	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
<i>Schizotaenia sigmodontis</i>	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
<i>Hymenolepis diminuta</i>	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
<i>Protospirura muris</i>	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
<i>Longistriata adunca</i>	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
<i>Syphacia sigmodontis</i>	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
<i>Strongyloides sigmodontis</i>	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 2. Indices of species richness (mean number of helminth species per host animal) and community diversity of gastrointestinal helminth communities of hispid cotton rats inhabiting the Oklahoma Refining Co. Superfund waste site, Caddo County, Oklahoma, from September 1993 to October 1995.

Treatment and Index	September 1993		February 1994		September 1994		April 1995		September 1995	
	Mean	S E	Mean	S E	Mean	S E	Mean	S E	Mean	S E
Reference										
Species richness	3.25	0.20	3.36	0.26	3.29	0.18	2.96	0.19	3.08	0.23
Community diversity	0.23	0.08	0.53	0.03	0.38	0.02	0.52	0.02	0.57	0.05
Contaminated										
Species richness	2.63	0.13	2.43	0.17	2.87	0.12	2.29	0.16	2.14	0.15
Community diversity	0.43	0.06	0.45	0.11	0.38	0.15	0.42	0.13	0.60	0.05

Table 3. Mean relative population densities (average number of individuals per 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.

Sites and dates	Total macroarthropods		Total insects		Class Hymenoptera		Class Coleoptera		Total arachnids		Order Araneae	
	Mean	S E	Mean	S E	Mean	S E	Mean	S E	Mean	S E	Mean	S E
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 4. Mean population densities (individuals $\times 10^3/m^2$) 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		Suborder oribatida		Suborder actinedida		Suborder gamasida	
	Mean	S E	Mean	S E	Mean	S E	Mean	S E	Mean	S E	Mean	S E	Mean	S E
Reference	12.3	1.7	5.2	1.1	1.9	0.6	6.9	0.9	4.8	0.6	1.3	0.4	0.5	0.1
Contaminated	13.1	1.3	3.3	0.6	2.1	0.5	9.4	1.1	6.1	0.8	2.1	0.4	0.7	0.2

Figure 1: Seasonal prevalences of cestode species collected from cotton rats on the three contaminated and two reference sites on the Oklahoma Refining Co. Superfund Waste Site, Caddo Co., 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, $P \leq 0.05$).

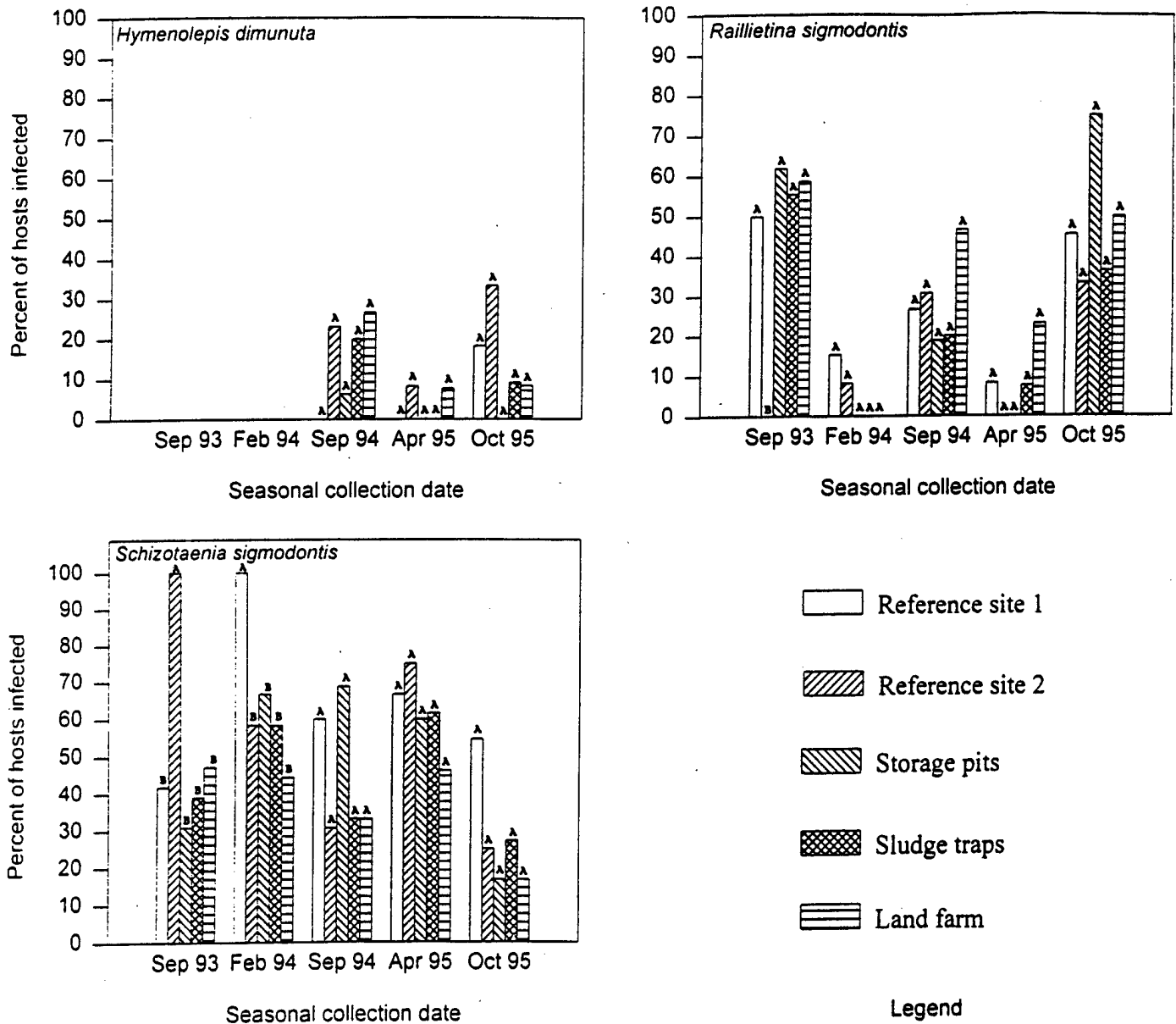


Figure 2: Seasonal prevalences of nematode species collected from cotton rats on the three contaminated and two reference sites on the Oklahoma Refining Co. Superfund Waste Site, Caddo Co., Oklahoma, August 1993 to October 1995. Different letters above the bars denote significant differences in prevalence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

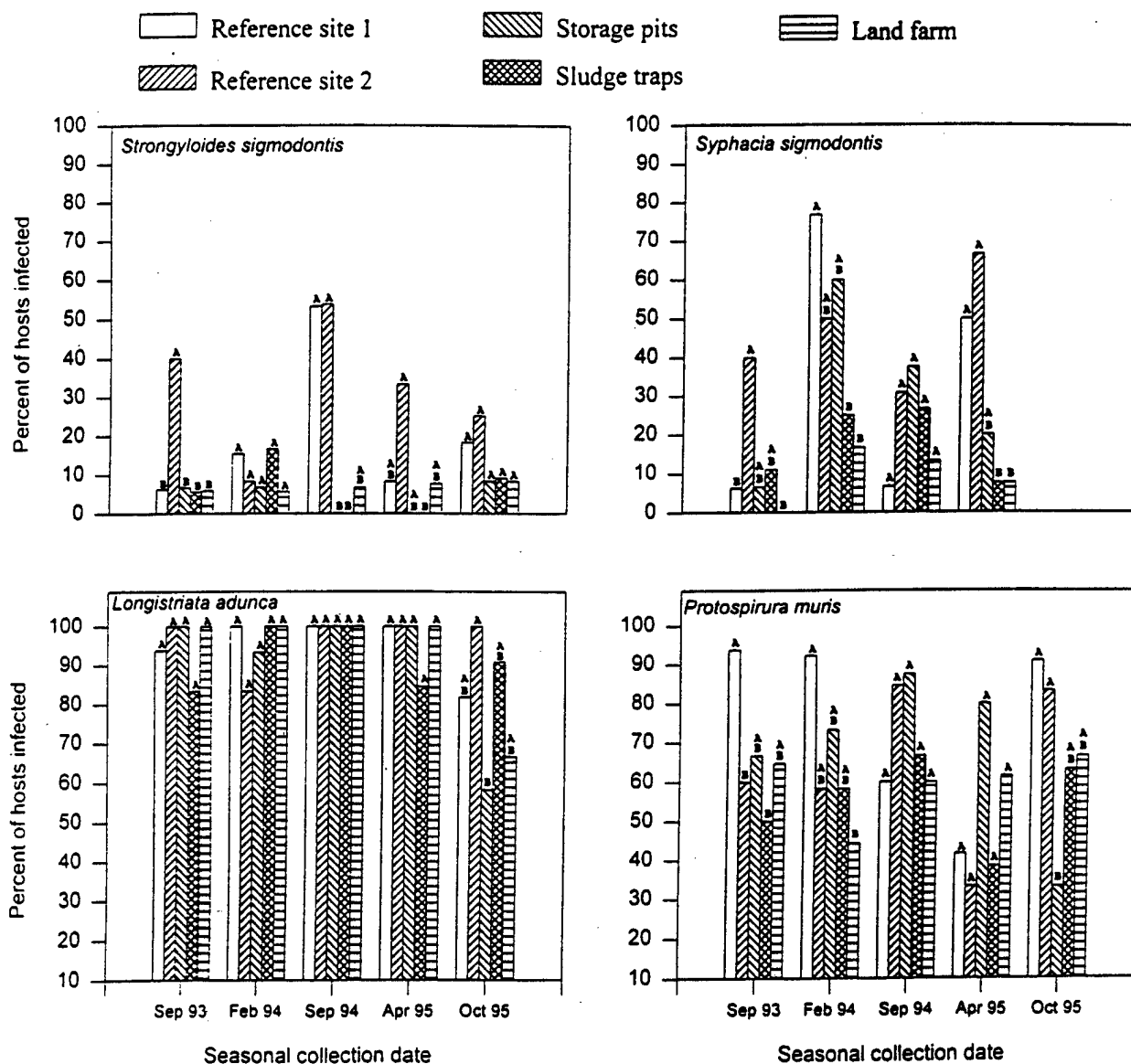


Figure 3: Single-linkage cluster diagrams depicting the similarity (Horn 1966) of helminth communities surveyed on three contaminated and two reference sites on the Oklahoma Refining Company superfund waste site, Caddo County, Oklahoma, August to October 1995.

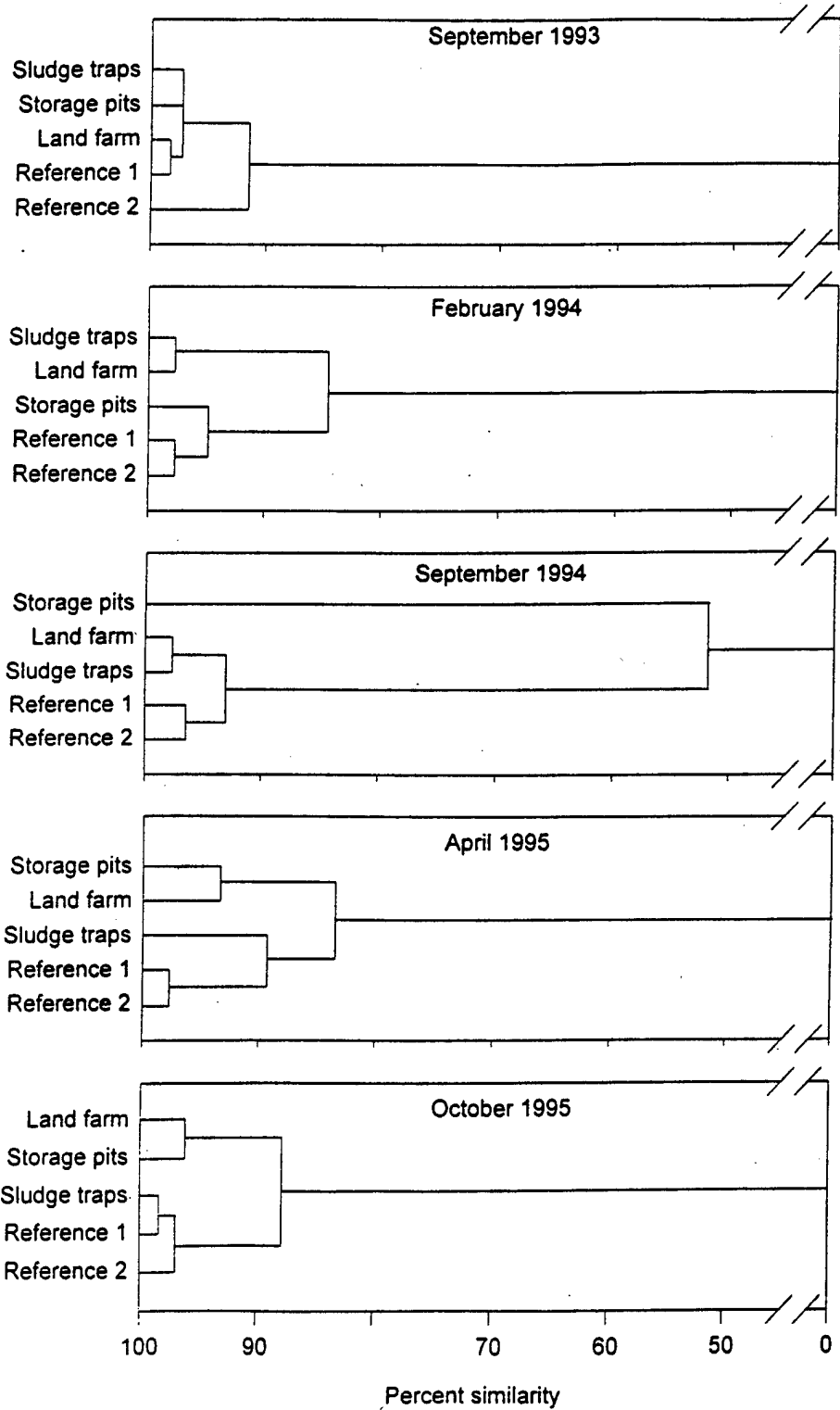


Figure 4: Relative densities (average number of individuals per trap) of terrestrial isopods recovered from contaminated and reference sites on the Oklahoma Refining Company superfund site, Caddo County, Oklahoma, August to October 1995.

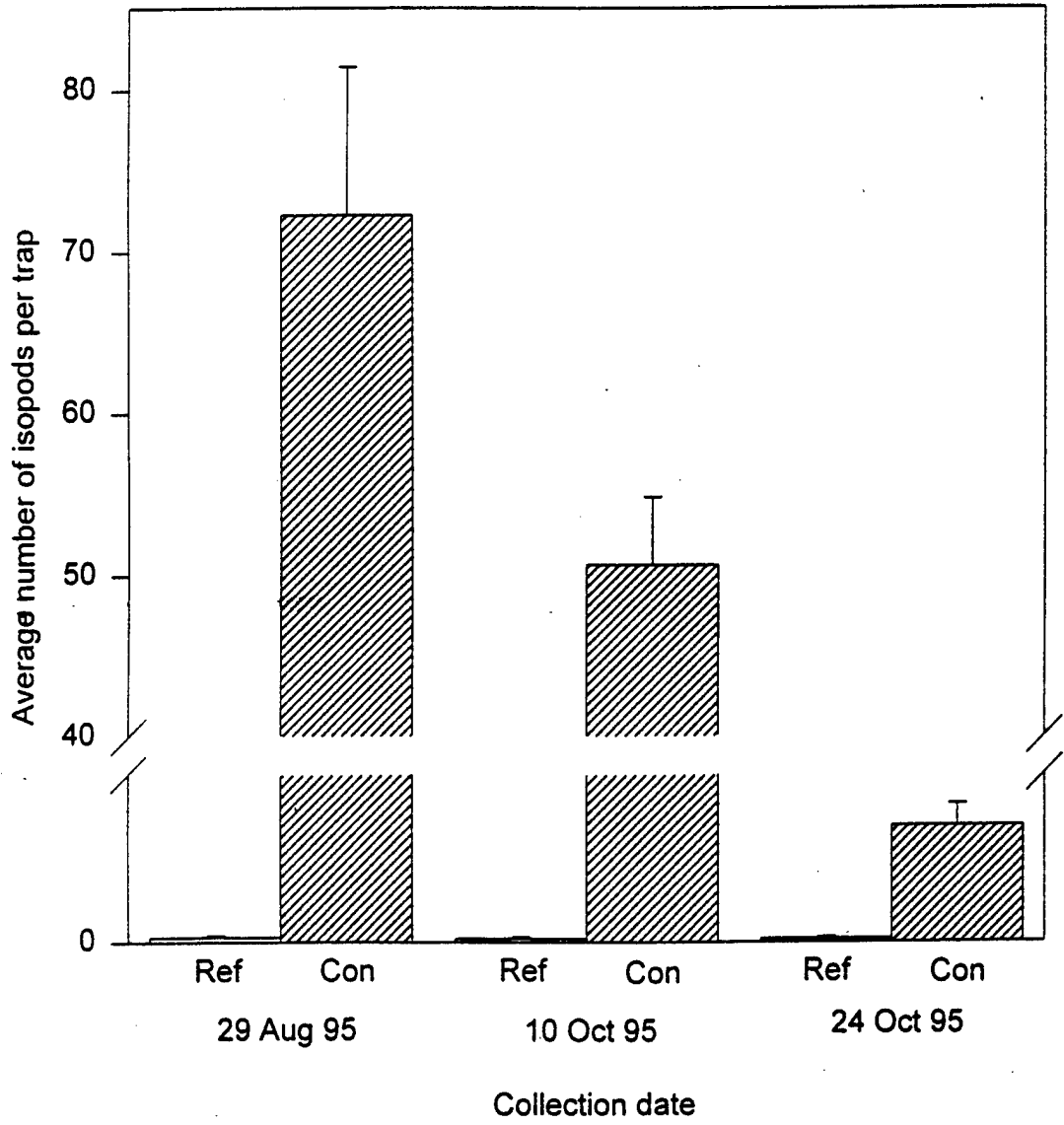
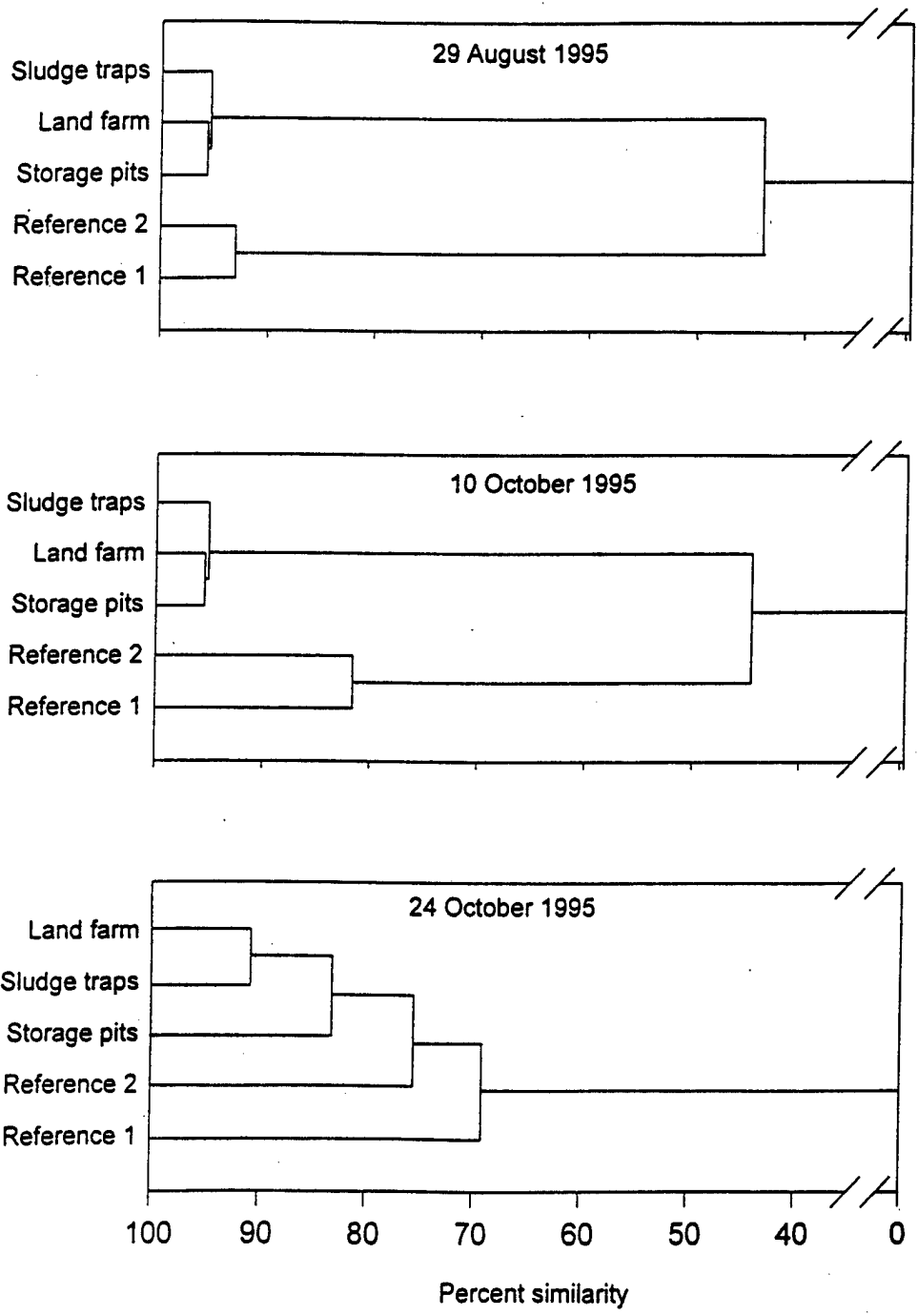


Figure 5: Single-linkage cluster diagrams depicting the similarity (Horn 1966) of macroarthropod communities surveyed on three contaminated and two reference sites on the Oklahoma Refining Company superfund waste site, Caddo County, Oklahoma, August to October 1995.



REPORT DOCUMENTATION PAGE

AFRL-SR-BL-TR-98-

0137

Public reporting burden for this collection of information is estimated to average 1 hour per response, including gathering and maintaining the data needed, and completing and reviewing the collection of information, collection of information, including suggestions for reducing this burden, to Washington Headquarters, Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Pa

a sources,
ect of this
Jefferson

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE		3. REPORT TYPE AND DATES COVERED FINAL 01 Jul 93 to 30 Jun 97	
4. TITLE AND SUBTITLE AN INEXPENSIVE MODEL SYSTEM FOR IN SITU EVALUATIONS OF ECOLOGICAL RISK IN PETROCHEMICAL-CONTAMINATED TERRESTRIAL ENVIRONMENTS				5. FUNDING NUMBERS F49620-93-1-0398 3484/YS 61103D	
6. AUTHOR(S) DR ROBERT L. LOCHMILLER					
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) DEPT OF ZOOLOGY OKLAHOMA STATE UNIVERISTY STILLWATER OK 74078				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) AFOSR/NL 110 DUNCAN AVE ROOM B115 BOLING AFB DC 20332-8050 DR WALTER J. KOZUMBO				10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES					
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited.				12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words) The overall objective of this research was further elucidate the mechanisms by which petrochemical contaminants influence terrestrial ecosystems and to develop reliable in situ biomonitoring tools for assessing risks to mammalian systems and ecosystems. Specifically, we approached this objective by examining the feasibility of using internal parasite communities harbored by resident small mammals as an in situ assay system for evaluating impacts of petrochemical contamination on complex ecological processes in terrestrial environments. Host parasite community ecology represents a culmination of a myriad complex ecological processes, including interactions among hosts, intermediate hosts, and parasites, host immunity, direct toxicity to parasites and other processes. Overall effects of petrochemical soil contamination on the structure (species composition and abundances) of the parasitic communities of cotton rats were evident in the single-linkage cluster analysis of similarity indices. 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. Higher relative densities of surface-dwelling macroarthropod groups were observed on the contaminated sites. The most notable differences					
14. SUBJECT TERMS				15. NUMBER OF PAGES	
				16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT (U)		18. SECURITY CLASSIFICATION OF THIS PAGE (U)		19. SECURITY CLASSIFICATION OF ABSTRACT (U)	
				20. LIMITATION OF ABSTRACT (UL)	

was the terrestrial isopods, which had densities 180 times higher on contaminated than reference sites. Significant differences were also noted in macroarthropod diversity, and the results of similarity comparisons of macroarthropod communities residing on the contaminated and reference sites indicated community abundances and composition on the contaminated sites were more similar to one another than to the communities residing on the reference sites. The results of our survey suggest that helminth parasite communities of cotton rats and macroarthropod communities in the habitat could be valuable ecosystem-level bioindicators for evaluating the effects of soil contamination on terrestrial systems.