BIOACCUMULATION OF TRACE ELEMENTS AND REPRODUCTIVE EFFECTS IN DEER MICE (*Peromyscus maniculatus*).

Andrea J. La Tier[1], Pamela I. Mulligan[2], Robert A. Pastorok[1], and Thomas C. Ginn[1]

Abstract: Reproductive parameters were used to evaluate the potential effects of exposure of female deer mice to trace elements-enriched soils in the Upper Clark Fork River Superfund Complex. Deer mice (*Peromyscus maniculatus*) were collected from floodplain (riparian) and transitional area habitats in each of four drainages with a wide range of trace element concentrations in soil. Whole-body tissue concentrations of As, Cd, Cu, Pb, and Zn were measured in composite samples of male and female deer mice. Females were necropsied and the uteri were removed to evaluate reproductive performance based on percentages of females with embryos and with uterine scars (i.e., implantation sites) and the mean numbers of embryos and uterine scars per female. Reproductive activity was evidenced by the presence of embryos or uterine scarring in animals from trace elements-enriched areas. Deer mice were prevalent in all areas and mean litter size (4.4 per female) was normal for this region (4–6 pups per female). These results indicate that elevated trace element concentrations in deer mice do not result in measurable effects on reproduction.

Additional Key Words: trace elements, bioaccumulation, reproductive effects.

Introduction

The Clark Fork River Superfund site comprises 26 operable units, which contain a wide range of habitat types. This investigation focused on riparian habitats and upland areas adjacent to riparian habitats (herein referred to as transitional areas) within three subregions of the Upper Clark Fork River Basin: Silver Bow Creek (SBC), Warm Springs Creek (WSC), and the Upper Clark Fork River (UCFR) (Fig. 1). Tailings were fluvially deposited in the SBC and UCFR subregions as a result of mining activities over the past 110 years. These activities have resulted in heterogeneous distribution of As, Cd, Cu, Pb, and Zn in soil (Table 1). Historical smelting activities in the WSC subregion have resulted in a homogeneous distribution of elevated soil trace element concentrations.

Small mammals are exposed to trace elements through numerous pathways including incidental soil ingestion (Beyer et al., 1994) as a result of preening and burrowing and consumption of plants and invertebrates that contain elevated tissue trace element concentrations. Trace elements such as As accumulate in plant tissue with a substantial portion translocated to seeds (Kabata-Pendias and Pendias, 1992).

Once exposed, the level of bioaccumulation is dependent on the mode of action of the trace element and the physiological response of the organism. It has been shown that Cd is highly mobile and readily bioconcentrates in the terrestrial food chain (Martin and Coughtrey, 1976; Martin et al., 1976, as cited in Roberts and Johnson, 1978; Van Hook, 1974, as cited in Roberts and Johnson, 1979). In mammals, Cd accumulates in the soft tissues with less than 10 percent of the total body burden being stored in the skeleton. Essential elements including Cu and Zn are generally better regulated than nonessential elements such as As, Cd, and Pb. The ability of higher animals to regulate the absorption and retention of Zn is well known and probably reflects the essential role of Zn in biological systems (Vallee, 1959, as cited in Roberts and Johnson 1978). Lead is not readily passed up the food chain of receptors such as raptors because the majority of the total body burden is deposited in skeletal components.

[1] PTI Environmental Services, Bellevue, Washington

[2] BioSystems Analysis, Santa Cruz, California (current affiliation: Trax Environmental Consulting, Bozeman, Montana)

Proceedings America Society of Mining and Reclamation, 1995 pp 3-14
DOI: 10.21000/JASMR95010003

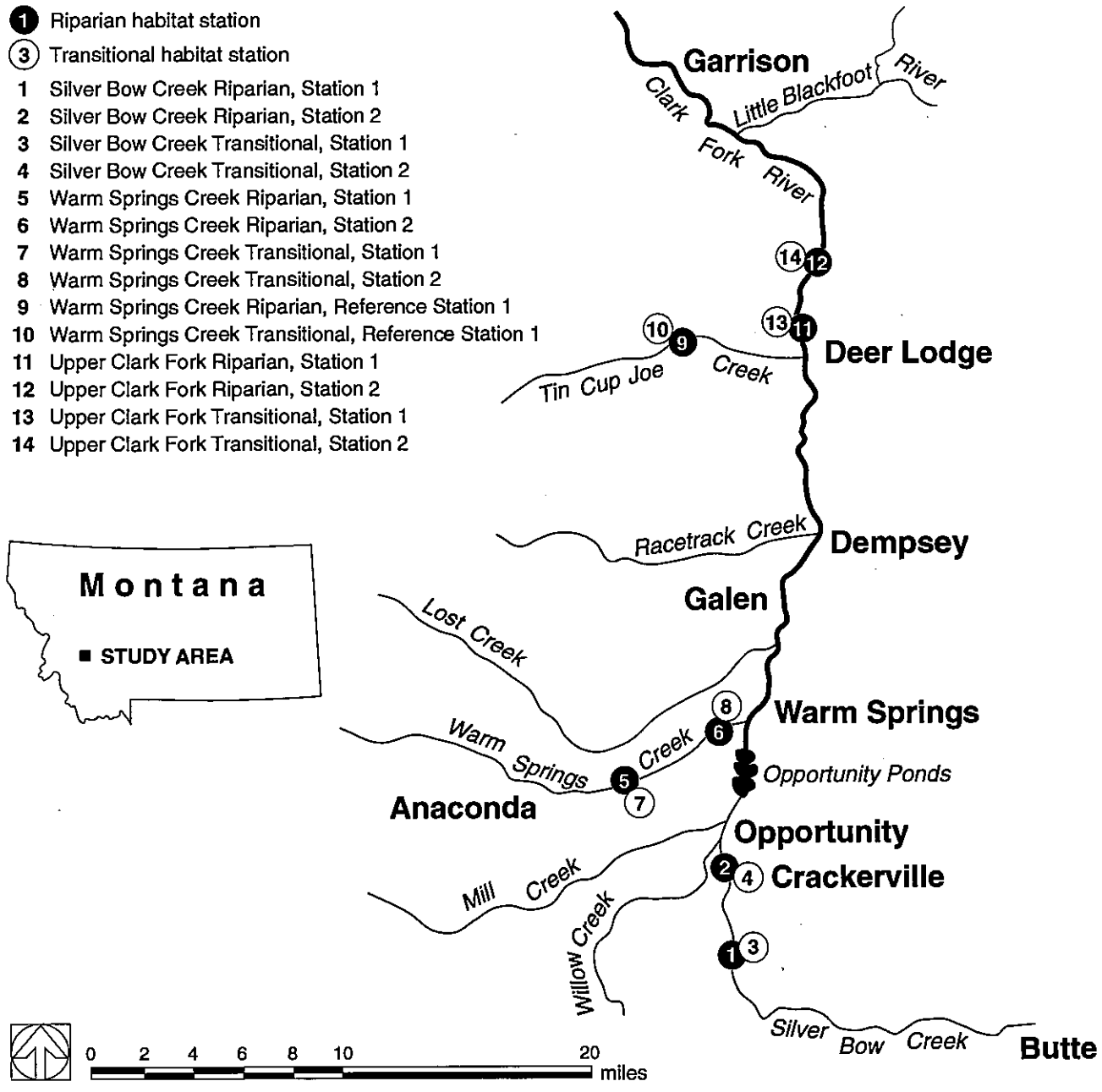

Figure 1. Mammal sampling stations.

Table 1. Deer mouse (*Peromyscus maniculatus*) trapping summary

Subregion	Habitat	Catch/Unit Effort[a]	Male/Female Ratio	Total Animals
SBC	Riparian	13.9	1.37	
	Transitional	10.7	1.03	
	Total			**542**
WSC	Riparian	13.7	1.18	
	Transitional	10.6	1.27	
	Total			**583**
UCFR	Riparian	3.2	0.97	
	Transitional	10.3	0.69	
	Total			**325**
WSCRF	Riparian	8.1	0.98	
	Transitional	8.6	1.34	
	Total			**200**

Note: SBC - Silver Bow Creek
UCFR - Upper Clark Fork River
WSC - Warm Springs Creek
WSCRF - Warm Springs Creek Reference Area (Tin Cup Joe Creek)

[a] Expressed as number of individuals caught per 100 trap nights.

The purpose of this study was to evaluate whether exposure to elevated soil trace element concentrations was affecting fertility of deer mice (*Peromyscus maniculatus*). The specific objectives included the following:

- Determining if female deer mice in any of the three subregions are experiencing reproductive impairment
- Determining the prevalence of reproductive impairment in deer mice from a reference area
- Determining if female deer mice from riparian and transitional habitats exhibit similar prevalence of reproductive impairments
- Determining if female deer mice from different subregions exhibit similar prevalence of reproductive impairments.

The data on effects in small mammals (e.g., gross abnormalities and reproductive effects) were used to directly assess risk to small mammals.

Methods

Potential reproductive effects in the deer mouse were evaluated by direct counts of the number of embryos and the number of implantation scars in the uteri of females. The general sampling design for the small mammal bioaccumulation and reproductive effects component is shown in Figure 2. In each subregion, two sampling stations were located within each of the two habitat types (i.e., near-stream riparian and transitional) to represent low and high concentrations of trace elements in soil. For each habitat type, a reference area station also was sampled.

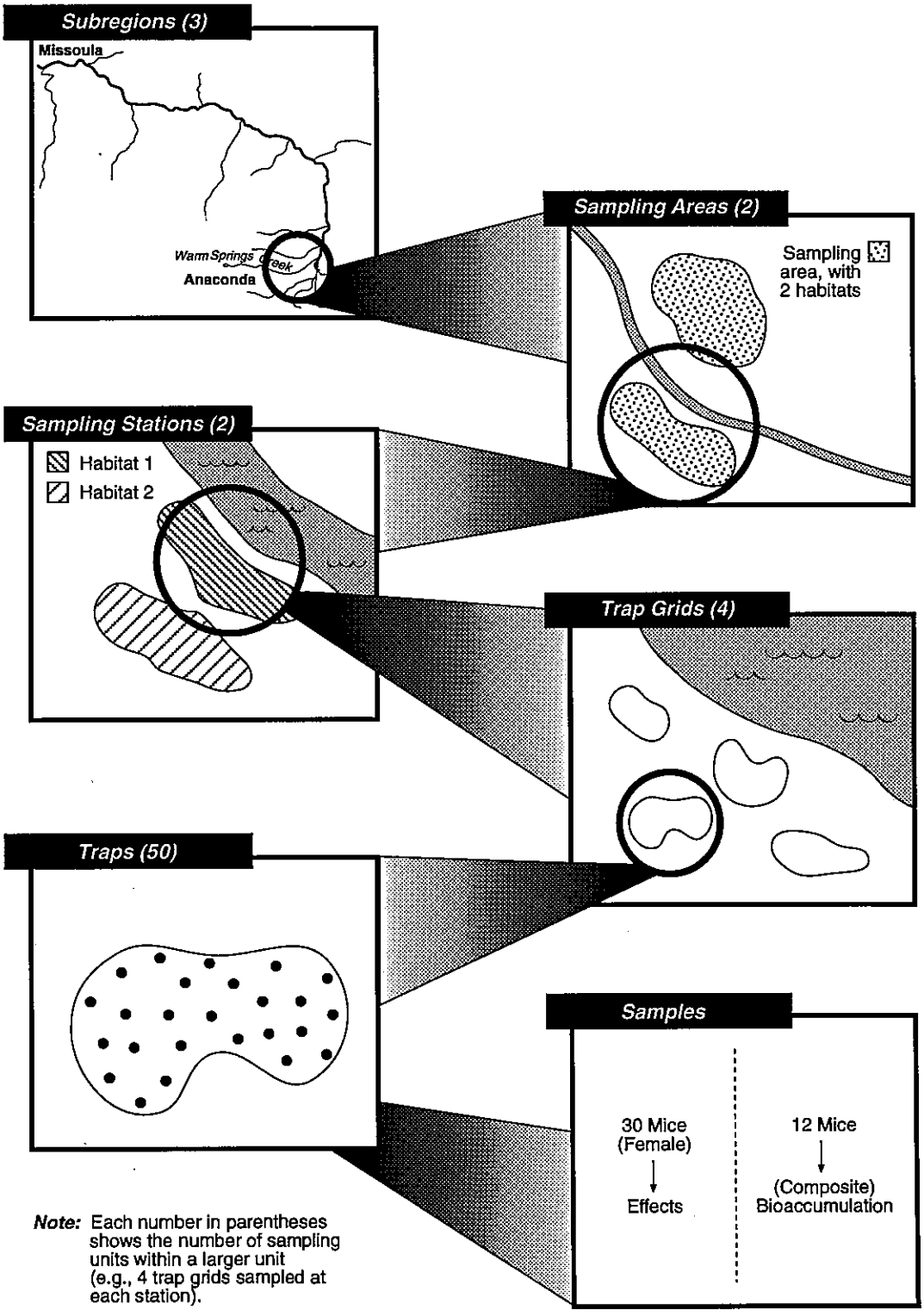

Figure 2. Schematic sampling design for mammal bioaccumulation and reproductive effects analysis.

Sampling Stations

Selection of potential sampling areas was based on inspection of historical data for soil trace element concentrations and on exposure/aspect information from topographic quadrangles. Sampling stations are shown in Figure 1. The transitional areas were sampled to evaluate the spatial distribution of mice with elevated body burdens of trace elements because individuals caught in transitional areas could have traveled to riparian areas where they might have been exposed to elevated trace element concentrations in soil, invertebrates, and vegetation. The near-stream riparian and transitional habitats sampled for mammals were differentiated in the field by considering 1) the relative proportions of obligate and facultative wetland plant species and the proportion of facultative upland species and 2) evidence of hydrological influences. Within a habitat, all stations were as similar as possible with respect to physical habitat parameters (i.e., topography, geology, and general soil characteristics). Sampling stations were designated with either T1 or T2, or R1 or R2 indicating that they were placed in transitional or riparian habitats. Reference area stations (T1, R1) were identified as WSCRFR1 and WSCRFT1.

Sampling Design

The general design for this study component is illustrated schematically in Figure 2. Small mammal sampling was conducted in each of the three subregions and in a single reference area. Within each subregion, four sampling stations (T1, T2, R1, R2), each consisting of four trap grids, were set up. Both male and female mice were collected for trace element bioaccumulation analysis. Each field sample consisted of multiple mice (minimum of 12 adults corresponding to 3 per trap grids at a station). Only adults were analyzed to minimize variability attributable to age-specific bioaccumulation patterns. Sampling for reproductive effects occurred at the same stations as the small mammal trapping for the bioaccumulation component. An attempt was made to obtain 30 adult female mice per trap grid (equaling 120 mice per station) for use in this analysis.

Sampling Procedures

At each small mammal station shown in Figure 1, four trap grids were sampled. Fifty Sherman live traps were set out in each trap grid. Traps were placed in microhabitats to maximize trap success and to best sample the specified area. Generally, all traps were placed at least 15 m apart. Every effort was made to place grids in transitional habitat greater than or equal to 1 km from grids in riparian habitat to minimize the chance of trapping the same local population in the two habitats. In cases where land access was limited and this criterion could not be met, the distance between grids in different habitats was maximized.

Traps were baited in the evening and checked the following morning. Upon capture, information including sex, age class, weight, and trap location was recorded for each individual mammal. The first three adult deer mice (weighing 10 g or more) trapped per grid were sacrificed and placed in a composite sample for the station (12 individuals total). Exceptions to this procedure were made when trap success was low. Deer mice were sacrificed by asphyxiation with ether. After sacrifice, each individual was placed in a separate plastic bag, and placed in ice chests with prefrozen, refreezable ice packs. Mice were transferred to laboratory freezers at the end of the field day. Deer mice remained in the freezer until shipped to Columbia Analytical Services in Kelso, Washington, or the laboratory responsible for homogenizing samples. In order to increase sample size, in some cases it was necessary to analyze female mice designated for bioaccumulation analysis for reproductive effects prior to shipment to the laboratory.

Laboratory analyses were conducted in accordance with Contract Laboratory Program statement of work 788 (U.S. EPA, 1988). The mammal tissue samples were prepared according to a modified Montana State laboratory procedure in which the samples were homogenized, freeze-dried, then digested with concentrated nitric acid (CAS, 1990).

Mammal Reproductive Effects

Female deer mice were dissected, and the thoracic and abdominal viscera were visually examined. All uterine scars, embryos, or signs of resorbing embryos were counted, and any special features (e.g., uterine bands, relative lightness or darkness of scars) were noted.

Results

Trapping Success

Deer mice appeared to be prevalent in all subregions except UCFR as indicated by a high trapping success (Table 1). Catch per unit effort (CPUE) ranged from 3.2 to 13.9 animals per 100 trap nights. Stations UCFRR1 and UCFRR2 in the UCFR subregion had 3.5 and 2.9 animals per 100 trap nights, respectively.

Reproductive Effects

The mean litter size for deer mice collected during this study (4.4 per female) was within the regional range (4–6 per female) for this species (Kirkland and Layne, 1989) (Fig. 3). These results suggest that the observed trace elements enrichment in the habitat of the deer mouse had no effect on reproduction (Table 2).

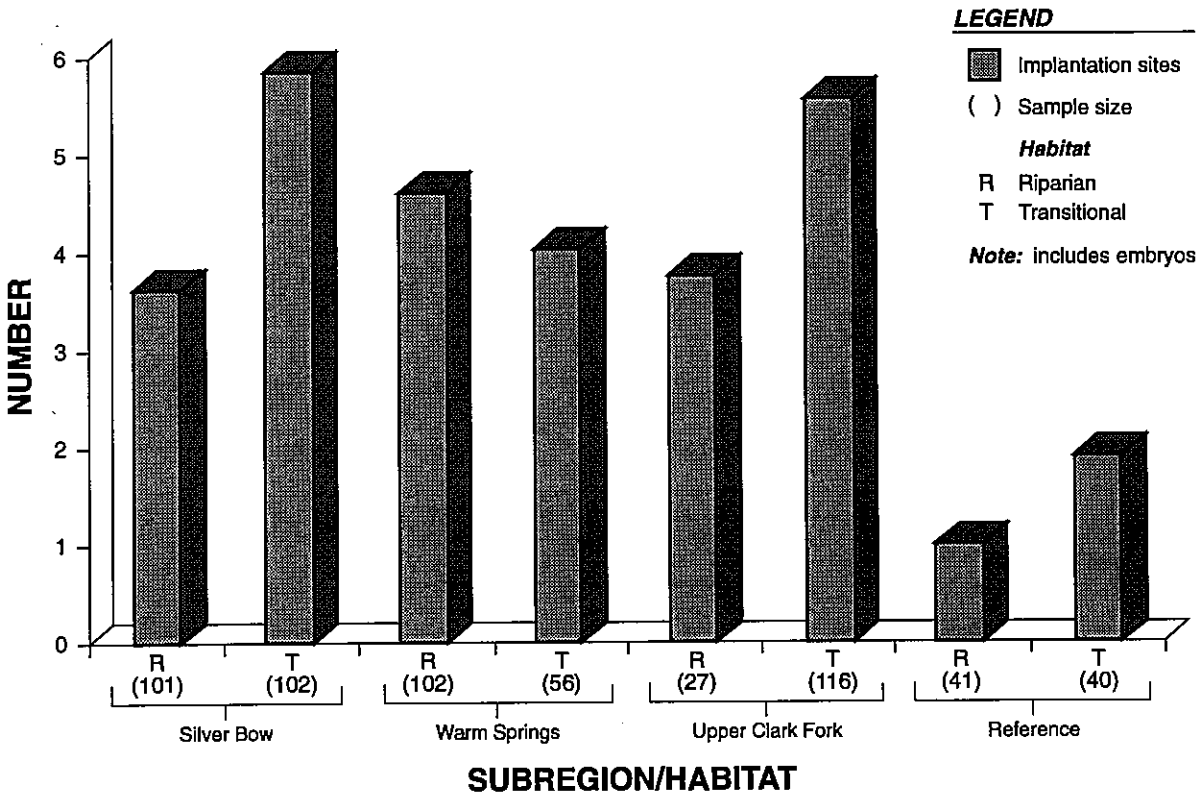

Figure 3. Mean number of implantation sites per female in deer mice (*Peromyscus maniculatus*).

Table 2. Average trace element concentrations (mg/kg dry weight) in soil samples at small mammal trapping stations

Station	As	Cd	Cu	Pb	Zn
SBCR1	201	2.8	529	399	1,180
SBCR2	217	3.1	817	577	1,164
SBCT1	178	2.2	193	68	148
SBCT2	278	11.1	1,190	776	1,992
WSCR1	586	6.6	1,730	326	788
WSCR2	130	4.0	199	167	158
WSCT1	1,400	4.6	1,200	491	817
WSCT2	154	3.6	311	122	322
WSCRFR1	42.2	0.32	27.0	7.8	65.1
WSCRFT1	63.7	1.58	118	37.3	142
UCFRR1	342	5.1	1,790	265	1,370
UCFRR2	452	5.5	2,329	386	1,659
UCFRT1	30	1	70	30	80
UCFRT2	32.1	0.75	73.1	22.9	82.2

Note: SBC - Silver Bow Creek
UCFR - Upper Clark Fork River
WSC - Warm Springs Creek
WSCRF - Warm Springs Creek Reference Area

None of the females necropsied from the reference area had embryos, and only 20 percent showed implantation scars. The majority of deer mice collected from the reference stations weighed between 10 and 15 g. Deer mice generally become reproductively active at weights greater than 15 g (Mulligan, 1993, pers. comm.). The animals collected from this area were young animals dispersing from the primary population into a new habitat. The low number of placental scars and lack of embryos from the reference stations may be age-related.

The order of stations with adult females with increasing numbers of implantation sites in the study area is as follows: WSC reference area riparian and transitional, SBC riparian, UCFR riparian, WSC transitional, WSC riparian, UCFR transitional, and SBC transitional (Fig. 3). Deer mice from the UCFR and WSC subregions had the greatest percentage of embryos followed by the SBC subregion.

Discussion

Trapping Success

Populations of the deer mice and, therefore, CPUE are dependent on habitat requirements such as quality and quantity of food, availability of water, density and distribution of nest sites, structure of living and dead vegetation, and depth and density of litter (Kirkland and Layne, 1989). These habitat requirements appear to be satisfied in all the subregions except UCFR regardless of soil contamination levels. This is evidenced by the high CPUE in the SBC subregion.

The lower trapping success at Station UCFRR1 was likely the result of a marginal deer mouse habitat. Deer mice are found in upland areas with dense litter and shrub overstory. They do not necessarily require dense grass, but prefer areas with shrubs as they have been documented to be arboreal foragers, consuming fruit and seeds and spending up to one-third of their time off the ground (Dewsbury et al., 1980). Two of the four grids at this station were located in areas that may have been too wet, while the other two were in an area that was probably heavily grazed.

The CPUE for deer mice at Station UCFRR2 was low probably as a result of the isolated nature of the habitat. This station was bordered to the north, south, and west by the Clark Fork River and to the east by a railroad berm (approximately 20 ft high). Although the shrub and grass cover provided good habitat for the deer mouse, these physical barriers effectively isolated the population. This isolation of the primary population may have reduced or eliminated emigration of juveniles into empty suitable habitats to form secondary populations and thereby regulated reproduction in the primary population resulting in a low CPUE.

Reproductive Effects

Deer mice from all subregions appeared to be reproductively active based on the prevalence of embryos and uterine implantation scars and on the mean numbers of embryos and implantation scars per female (Fig. 3 and 4). In addition to embryos, the presence of implantation sites or placental scars on the uterus provides evidence of pregnancy in small mammals (Mihok, 1987). The low number of placental scars

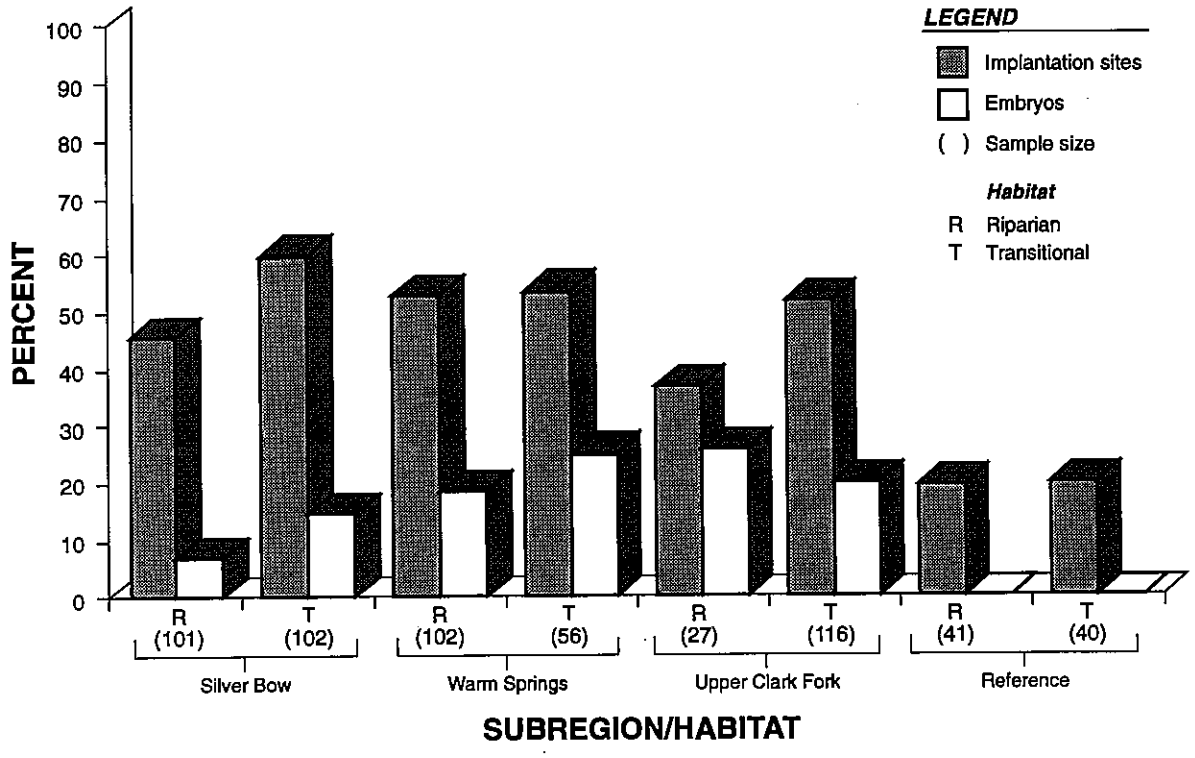

Figure 4. Percent of deer mouse (*Peromyscus maniculatus*) females with implantation sites and embryos.

and lack of embryos from the WSC reference stations may be age-related. None of the female mice trapped at the WSC reference stations had embryos, and the percentage of females with uterine scars (implantation sites) was lower than at any of the other subregions (Fig. 3). Most individuals (male and female) weighed 10–15 grams and appeared to be subadults. Rarely was a juvenile or larger adult captured. The age and size distribution of the mice at this site was unique among the subregions. The WSC reference area may have been a population sink for dispersing juveniles. A population sink is a habitat patch where individuals are able to survive but not reproduce because of some limiting environmental factor. Population sinks are colonized by individuals from a reproducing source population in a more suitable habitat patch (Wiens, 1985).

Exposure to elevated soil trace element concentrations does not appear to inhibit reproduction in deer mice. Physiologically nonregulated trace elements such as Cd, As, and Pb are not bioaccumulated to the extent at which reproduction is adversely affected (Fig. 3–5).

The intake of trace elements is likely to be less than the literature derived toxicity reference values (TRVs) for reproductive effects. For instance, data for As from chronic feeding studies in the literature support TRVs of 1–4.2 mg/kg-day for reproductive effects in mice (Schroeder and Mitchener, 1971; Hood et al., 1987 [results of an unpublished study]). The lowest-observed-adverse-effect level (based on reproductive endpoints) for dietary Pb (100 mg/kg dry weight) was calculated to be equivalent to a daily intake of 30–60 mg/kg for shrews and wild rodents (Shore and Douben, 1994). The effects of Zn ingestion by small mammals have been reviewed by ATSDR (1992). Ingestion of 200 mg/kg-day of zinc oxide before mating and during gestation increased fetal resorption in mice (Schlicker and Cox, 1968). Reproductive failure was observed when both male and female mice were exposed to 1.9 mg/kg-day Cd in drinking water (Schroeder and Mitchener, 1971). Reproductive effects, such as increased fetal mortality, were observed in pregnant mice fed 104 mg/kg-day copper sulfate; fetal mortality or developmental abnormalities were not observed at dose rates of 78 mg/kg-day (Lecyk, 1980).

Implantation scars do not indicate whether an embryo was resorbed, aborted, or born. However, the numbers of animals trapped suggest that deer mice are maintaining populations at levels comparable to reference sites with higher levels of reproductive activity.

Conclusions

The deer mice in all subregions appeared to be fertile. Numbers of embryos per female were consistent with typical litter size (4–6 pups, mean equal to 4.4; Kirkland and Layne, 1989) for this species. The percentage of females with implantation sites and the percentage of females with embryos were higher at study sites than in the reference area. The relative abundance of the deer mouse was generally high at study sites compared with the reference area. Based on these indicators, trace elements enrichment in the Clark Fork River Basin appears to have no adverse effect on deer mouse abundance and reproduction.

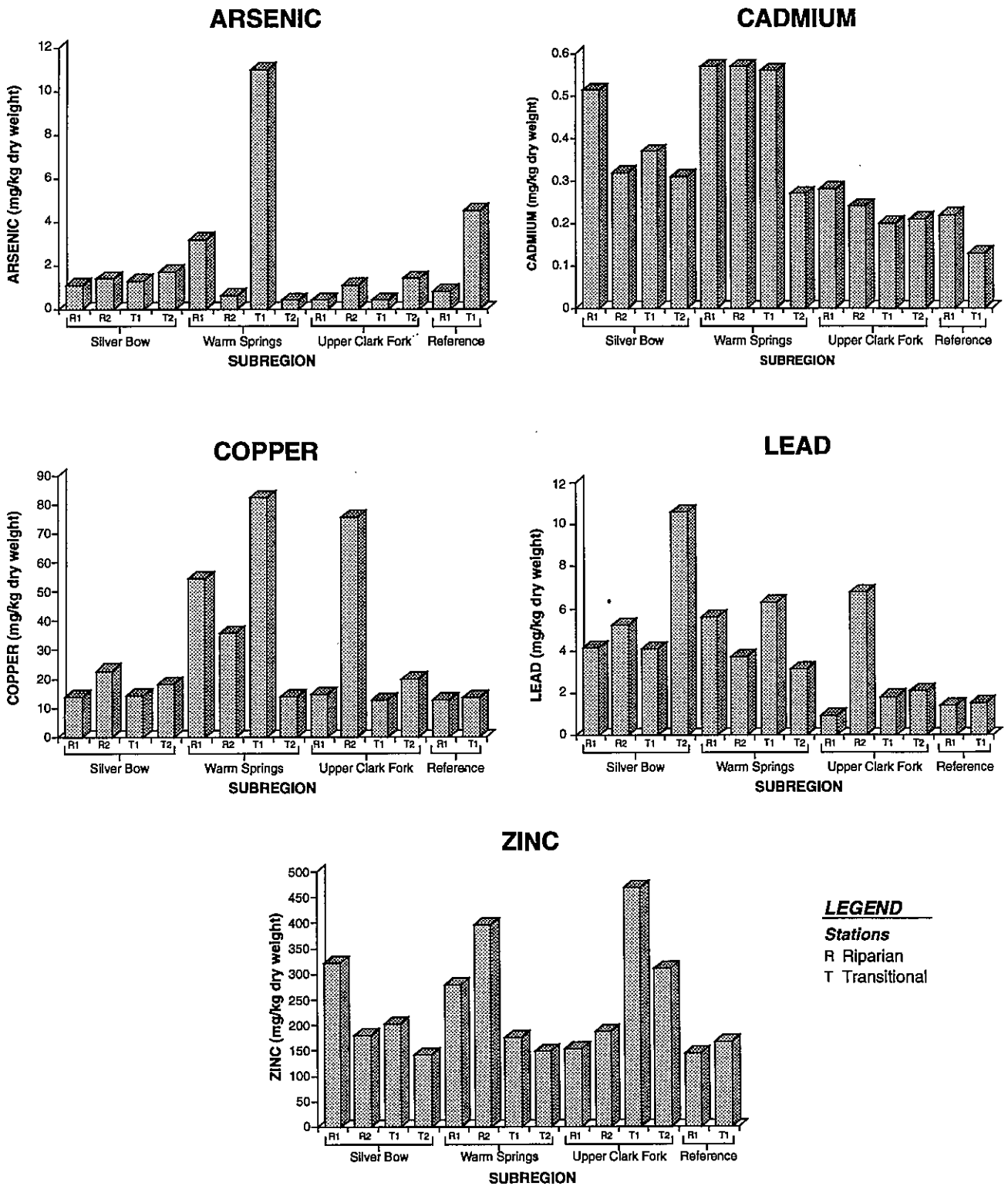

Figure 5. Trace element concentrations in deer mice (*Peromyscus maniculatus*) by station.

References

ATSDR. 1992. Toxicological profile for zinc. U.S. Department of Health and Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

Beyer, W.N., E. Connor, and S. Grould. 1994. Estimates of soil ingestion by wildlife. J. Wildl. Manage. 58:375–382.

CAS. 1990. Sample preparation of biological tissue for heavy metal analysis by AA or ICP. Columbia Analytical Services, Inc., Kelso, WA.

Dewsbury, D.A., D.L. Lanier, and A. Miglietta. 1980. A laboratory study of climbing behavior in 11 species of muroid rodents. Am. Mid. Nat. 103(1):66–72. http://dx.doi.org/10.2307/2425039

Hood, R.D., G.C. Vedel-Macrander, M.J. Zaworotko, F.M. Tatum, and R.G. Mecks. 1987. Distribution, metabolism, and fetal uptake of pentavalent arsenic in pregnant mice following oral or intraperitoneal administration. Teratology 35:19–25. http://dx.doi.org/10.1002/tera.1420350104

Kabata-Pendias, A., and H. Pendias. 1992. Trace elements in soil and plants. Second Edition. CRC Press, Boca Raton, FL.

Kirkland, G.L., and J.N. Layne (eds). 1989. Advances in the study of *Peromyscus* (Rodentia). Texas Tech University Press, Lubbock, TX. 366 pp.

Lecyk, M. 1980. Toxicity of cupric sulfate in mice embryonic development. Zool. Poll. 28:101–105.

Martin, M.H., and P.J. Coughtrey. 1976. Comparisons between the levels of lead, zinc and cadmium within a contaminated environment. Chemosphere 5:15–20 (not seen, as cited in Roberts and Johnson 1978). http://dx.doi.org/10.1016/0045-6535(76)90050-3

Martin, M.H., P.J. Coughtrey, and E.W. Young. 1976. Observations on availability of lead, zinc, cadmium and copper in woodland litter and the uptake of lead, zinc and cadmium by the woodlouse, *Oniscus asellus*. Chemosphere 5:313–318 (not seen, as cited in Roberts and Johnson 1978). http://dx.doi.org/10.1016/0045-6535(76)90005-9

Mihok, S. 1987. Pregnancy test for meadow voles (*Microtus pennsylvanicus*) based on blood azurocyte counts. Can. J. Zool. 65:2830–2832. http://dx.doi.org/10.1139/z87-430

Mulligan, P. 1993. Personal communication (telephone conversation with A. La Tier of PTI Environmental Services, Bellevue, WA, in December 1993). Biosystems, Inc., Bozeman, MT.

Roberts, R.D., and M.S. Johnson. 1978. Dispersal of heavy metals from abandoned mine tailings and their transfer through terrestrial food chains. Environ. Pollut. 16:293–310. http://dx.doi.org/10.1016/0013-9327(78)90080-0

Roberts, R.D., and M.S. Johnson. 1979. Predator-prey relationships in the food chain transfer of heavy metals. pp. 104–114. In: Proc. of University of Missouri Annual Conference on Trace Substances in Environmental Health, Columbia, MO. D.D. Hempill (ed).

Schlicker, S.A., and D.H. Cox. 1968. Maternal dietary zinc, and development and zinc, iron, and copper content of the rat fetus. J. Nutrition 95:287-294. https://doi.org/10.1093/jn/95.2.287

Schlicker, S.A., and D.H. Cox. 1968. Maternal dietary zinc, and development and zinc, iron, and copper content of the rat fetus. J. Nutrition 95:287–294.

Schroeder, H.A., and M. Mitchener. 1971. Toxic effects of trace elements on the reproduction of mice and rats. Arch. Environ. Health 23:102-106. http://dx.doi.org/10.1080/00039896.1971.10665963

Shore, R.F., and E.T. Douben. 1994. Predicting ecotoxicological impacts of environmental contaminants on terrestrial small mammals. Reviews of Environmental Contamination and Toxicology. Volume 134.

U.S. EPA. 1988. Contract Laboratory Program statement of work. Inorganic analysis, multi-media, multi-concentration, July 1988. SOW No. 788. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Las Vegas, NV.

Vallee, B.L. 1959. Biochemistry, physiology and pathology of zinc. Physiol. Rev. 39:443–490 (not seen, as cited in Roberts and Johnson 1978).

Van Hook, R.I. 1974. Cadmium, lead and zinc distributions between earthworms and soils: potentials for biological accumulation. Bull. Environ. Contam. Toxicol. 12:509–512. http://dx.doi.org/10.1007/BF01684990

Wiens, J.A. 1985. Vertebrate responses to environmental patchiness in arid and semiarid ecosystems. In: The ecology of natural disturbance and patch dynamics. S.T.A. Pickett and P.S. White (eds). Academic Press, Inc., San Diego, CA. pp. 169–193.