An Analysis of the Contamination by and Effects of Highway-Generated Heavy Metals on Roadside Stream Ecosystems

by

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(ABSTRACT)

This study examined the consequences of the opening and operation of a new highway north of Richmond, Virginia with respect to contamination of the aquatic environment with heavy metals (Zn, Cd, and Pb), and the effects of these metals on the biota of roadside streams. Sixteen sites located on six small, soft-water streams that were crossed by the highway, encompassing six reference sites located upstream of the highway, six sites located directly at the highway, and four sites located downstream of the highway, were sampled over a two and a half year period, allowing both spatial and temporal analyses.

Traffic densities on the highway averaged about 12,000 vehicles per day (vpd). Significant increases in the metals concentrations of sediment, benthic invertebrates, fish whole-bodies, and fish tissues (liver, kidney, and bone) were noted over the course of the study, although the in-

creases varied in magnitude, and were not always consistent. Sediment metals concentrations followed a dynamic plateau. Fish whole-body concentrations of Cd and Pb increased steadily over the course of the study. Spot-sampling for the same parameters along another nearby, more heavily travelled highway (50,000 vpd) indicated that increases in metals concentrations in the different ecosystem components at the study streams would have been greater had there been more traffic.

A number of biotic parameters were investigated to determine whether metals contamination was affecting the biological integrity of the study sites. These were: benthic macroinvertebrate diversity and density; the percentage of the aquatic insect community that was composed of chironomids; and fish community diversity, density, and biomass. Only benthos density, the percent chironomids, and fish species diversity showed changes that could be related to metals contamination. Indications from spot sampling along the more heavily travelled highway were that if more contamination had been experienced, more biotic parameters would have been disturbed, and to a larger extent. Fish community structure analyses using the Pinkham-Pearson coefficient of similarity indicated that fish community structure became increasingly altered at highway sites, and to a lesser degree downstream sites, over the study period.

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INTRODUCTION

With increasing urbanization, an increasing amount of our land is being covered with concrete and asphalt in the form of buildings, streets and parking lots in the cities, and increasingly crowded highways between cities. Airborne particulate emissions from automotive exhaust which in the past have fallen onto the soil and been trapped there now accumulate on these impervious surfaces until washed into nearby streams. A major component of these emissions is the lead added to gasoline in anti-knock compounds. Other heavy metals that have been identified in significant quantities in roadside dust include cadmium, from lubricating oil, and zinc, from oil and tires (Lagerwerff and Specht 1970). Although the toxicity of these metals to stream organisms in laboratory studies is well documented (U.S. Environmental Protection Agency 1976), the actual impact of these pollutants on the biota of the receiving streams remains largely unexplored (Van Hassel et al. 1980; Smith and Kaster 1983).

Lead has been the most studied of these three metals with regard to generation and dispersal by automotive traffic.

The magnitude of automotive lead emissions to the atmosphere is enormous. In 1970, an estimated 140 million kg of lead was emitted by vehicles in the U.S. alone (Wheeler et al. 1978). Ewing and Pearson (1974) estimated that automotive emissions constitute 95% of the total lead emissions to the atmosphere. Although the majority of these emissions occur in urban areas, it is estimated that 306,000 km^2 (roughly 3.3%) of the U.S. is within 50 m of highways with traffic densities of more than 1000 vehicles per day (Smith 1976).

Because of the regulation of lead content in gasoline by Congress in 1973, and the requirement that 1974 and later model automobiles be required to run on unleaded gasoline, expectations are that the amount of lead introduced into the environment has been decreasing in recent years. However, as of 1977, no assessment of the matter had been made (Friedlander 1977). Some complicating factors in the attempted lead reduction are the widespread noncompliance wih the law prohibiting the introduction of leaded gasoline into newer cars, recent high interest rates and car prices that have kept pre-1974 cars on the road longer than anticipated, and the growing numbers of cars on the road in general. Nonetheless, Byrd et al. (1983) found levels of lead in soils directly alongside a Louisiana highway in 1979 to average 35% of what they were in 1973, indicating to them

that roadside lead contamination may be decreasing. However, over the same time interval, the lead levels in soils 10m from the road surface increased an average of 150%, indicating to them a slow movement of metals through the soil to roadside streams.

There is no reason to expect that the amounts of zinc or cadmium entering the roadside environment will decrease. These metals differ from lead in the manner in which they are introduced into the environment. These metals, originating from dripped oil and abraded tires, enter the roadside environment mainly through highway runoff as a result of rainfall, rather than by a continuous aerial deposition. This results in slightly different patterns of accumulation (Rodriguez-Flores and Rodriguez-Castellon 1982).

Evidence of Roadside Metals Contamination

Heavy metals have been identified in elevated quantities in roadside dust (Revitt and Ellis 1980; Harrison et al. 1981; Ellis and Revitt 1982) and soils (Lagerwerff and Specht 1970; Motto et al. 1970; Ward et al. 1977; often in direct proportion to traffic density (Lau and Wong 1982; Rodriguez-Flores and Rodriguez-Castellon 1982). Elevated levels of these heavy metals have also been found in

vegetation bordering (Motto et al. 1970; Goldsmith et al. 1976), and invertebrates and mammals living near highways (Blair et al. 1977; Goldsmith and Scanlon 1977).

These metals are not restricted to the terrestrial environment surrounding highways, but find their way into nearby aquatic systems. Owe et al. (1982) found elevated levels of heavy metals in parking lot runoff. Getz et al. (1977) showed that a single Illinois watershed with an annual incoming load of 16,000 kg of lead from highways and other urban sources had an output of only 800 kg per year, with the remainder of the lead accumulating somewhere in the system. Van Hassel et al. (1980) found lead concentrations in the sediments of a second-order Virginia stream to be highly correlated with traffic density at bridge crossing sites. They also found that whole-body concentrations of lead in fish and invertebrates were significantly correlated with traffic density and equivalent to levels found in industrially-polluted streams.

Toxicity of Heavy Metals to Aquatic Organisms

Heavy metals are some of the most toxic of the elements. They are especially deleterious environmental pollutants because they cannot be degraded into a non-toxic state, as is the case with most organic pollutants. Consequently,

heavy metals tend to accumulate in the aquatic environment, especially in bottom sediments, from which they can be slowly released back into the water with changes in pH and ionic strength (Paul and Pillai 1983a; Tada and Suzuki 1982), or absorbed by aquatic invertebrates (Evans and Lasenby 1983), and fish (Delisle et al. 1975).

Although the mode of toxic action differs among metals and often with different concentrations of the same metal, all of the metals can affect enzyme systems by sulfhydryl binding, chelation, and salt formation (Jackim 1974). In addition to lethality, heavy metals can have a variety of sublethal effects on fishes. These include suppressed reproduction, delayed embryonic development, deformed vertebrae, morphological aberrations, and inhibition of growth (U.S. Environmental Protection Agency 1980a,b,c).

Laboratory Toxicity of Heavy Metals to Aquatic Life

There are two basic categories of laboratory tests designed to assess the toxicity of compounds to aquatic life. The first category of these bioassays are the acute toxicity tests. These tests are used to determine LC50s, or the concentration that will be lethal to 50% of the test organisms within a relatively short time period, usually 96 hours. The second category of aquatic toxicity tests are

chronic toxicity tests. These tests expose the organisms to generally lower levels of the test compound, but for extended periods, often a complete life cycle. These tests are designed to assess the long term chronic effects of chemicals on aquatic organisms. The end point in these tests is often not organism death, but other factors such as decreased growth, reproductive failure, and other non-lethal effects.

The results of both types of these bioassays have been used by the United States Environmental Protection Agency (EPA) to establish ambient water quality criteria for the protection of aquatic life. These criteria are formulated to specify concentrations of toxicants below which they pose no hazard to aquatic life. The criteria are developed taking many factors into account, and are ultimately based on the lowest effect concentration (LEC), which is the lowest concentration at which toxicity is noted, for the most sensitive species. In addition, when water quality parameters (e.g. hardness) can mediate toxicity, they are incorporated. The criteria also include a safety factor, to protect untested species. Two distinct types of criteria are developed: 1) a concentration never to be exceeded, which is based on the acute toxicity testing and is designed to prevent short term lethality; and 2) a 24-hour average

concentration which is not to be exceeded. The latter criterion is based on the chronic bioassays and is designed to prevent the more subtle long term effects of chronic, low level exposure. The 1980 EPA ambient water quality criteria for zinc, cadmium, and lead, expressed as a range, based on average hardnesses of streams in this study, and the LEC's are summarized in Table 1 .

Field Toxicity and Fate of Metals in Streams

To predict the environmental effects of a metal pollutant, one must consider the physico-chemical transformations that the metal undergoes after its release into the environment (Laxen and Harrison 1977; Freedman et al. 1980; Houba and Remacle 1982). Unfortunately, the chemistry of metals in aquatic environments is so complex that little is known with certainty. In fact several researchers (Laxen and Harrison 1977; Forstner and Prosi 1979) conclude that published reports are often contradictory. This complexity should be exacerbated in the case of automotive exhaust emissions, because they contain several different lead compounds (primarily lead halides), all of which can undergo transformations in the receiving system.

TABLE 1

1980 EPA Water Quality Criteria (ug/l) for Zn, Cd, and Pb[1]

	ZINC	CADMIUM	LEAD
Acute criteria	69-130	0.435-0.958	18.1-45.3
Acute LEC-fish	90	1.0	1170
Acute LEC-invertebrate	100	3.5	124
Chronic criteria	47	0.0036-0.0079	0.05-0.29
Chronic LEC-fish	47	1.7	19
Chronic LEC-invertebrate	47	0.15	12

[1] Criteria expressed as a range reflect the range of mean hardness found in the study streams: 16 to 34 mg/l as CaCO3.

Nonetheless, some generalizations can be made. Dissolved free metal ions are the most toxic, followed by complex ion entities, inorganic ion-pairs and complexes, organic complexes, metals sorbed on colloids, precipitates, and mineral particles (Freedman et al. 1980; Houba and Remacle 1982). The solubility of metals increases with increasing acidity. As a result, low pH favors aquated metal ions, high pH favors precipitates and co-precipitates. Increased water hardness favors the formation of inorganic complexes, precipitates and co-precipitates, at the expense of aquated ions (Hem and Durum 1973; Demayo et al. 1982). It can thus be concluded that the most susceptible streams to metals pollution would be soft-water acidic streams.

In many instances, toxicity occurs in streams that have metals concentrations judged by bioassays to be safe. One major reason for this is that fish in streams are often subjected to many contaminants at the same time. In these cases, their ability to withstand levels of any one toxicant may be lessened. This synergistic effect of many toxicants is well documented. Zinc in particular shows marked synergistic effects with other metals in acute toxicity experiments (Weatherly, et al. 1980). The result is that determinations of environmental effects cannot be made using only chemical data and the results of bioassays.

Conversely, fish and invertebrates are sometimes found thriving in waters with concentrations of metals judged by bioassays to be harmful (Stern and Walker 1978; Roch et al. 1982), and often in water that fails EPA ambient water quality criteria. Two factors help to explain this phenonemon. The first is that the chemistry of metals in aquatic systems is extremely complex (see Hem and Durum 1973) and each of the various metal species has its own toxicity (Houba and Remacle 1982; Weatherly et al. 1980). In addition, under certain conditions metal ions readily complex with other inorganic or organic constituents in the water and as a result become unavailable for uptake by the resident biota, or lose their biologic activity (Freedman et al. 1980; Houba and Remacle 1982). The other factor explaining fish survival at toxic concentrations of contaminants is acclimation. Fish have built-in defense mechanisms that allow them (within bounds) to acclimate to otherwise harmful levels of pollutants.

For instance, it has been shown that rainbow trout acclimated to sublethal levels of a heavy metal (copper) were able to withstand exposures of 1.7 times the incipient lethal level, whereas control fish (not pre-exposed) died rapidly (Dixon and Sprague 1981a). This suggests an internal mechanism for defense against heavy metals that is

induced by exposure. In mammalian systems metallothioneins, metalloderivatives of the sulfur-rich protein thionein, have been demonstrated to increase heavy metal tolerance (see Webb 1979). The presence of metallothionein in fish has been suspected (Brown et al. 1977) but until recently had never been analytically confirmed. Dixon and Sprague (1981b) isolated a low molecular weight protein that was induced by copper exposure. More recently, Ley et al. (1982) confirmed the identification of metallothionein in metals-exposed rainbow trout, Salmo gairdneri, and Roch et al. found the concentrations of metallothionein in rainbow trout livers to be positively related to environmental concentrations of heavy metals.

However, the ability of a fishes' defense mechanisms to resist harm from metals exposure cannot be limitless, and is as yet undetermined. Indications are that only a narrow range of metals concentrations may result in increased tolerance. Too low a concentration may sensitize the fish, while too high a concentration can reduce growth (Dixon and Sprague 1981a) or cause other sublethal effects. In addition, the ability of these defense systems to deal with combinations of metals has yet to be thoroughly examined, but may not be as great. For example, Dixon and Sprague (1981a) found that the level of exposure that afforded extra

tolerance to copper at the same time reduced the tolerance to zinc by 56%.

Documentation of Effects: Biomonitoring

The use of biomonitoring studies to determine effects of pollutants in the field is a relatively new practice. Early work was carried out on the effects of point-source organic enrichment and its resultant effects on dissolved oxygen concentrations. At present, there are several generally accepted techniques for identifying organic pollution of this kind, including diversity indices, similarity indices, biotic indices based on indicator species, and species abundance relationships (Cairns 1977; Washington 1984).

Biological monitoring to determine the existence and impacts of metals comtamination, on the other hand, is in its infancy. Many of the same techniques used for organic enrichment monitoring may be suitable for monitoring metals, however studies documenting their usefulness are few. A few studies question the usefulness of some techniques (particularly diversity) at all in metals pollution studies (e.g. Moore 1979; Chadwick and Canton 1984).

Winner et al. (1980) investigated insect community structure as an index of heavy metal pollution in two second-order Ohio streams. They found a predictable graded

response to metals pollution involving an increase in the percentage that chironomids comprised of the total aquatic insect community as metals pollution increased. Pratt et al. (1981) studied a Massachusetts stream receiving urban runoff and found that the macrobenthic community became increasingly disrupted (in terms of species diversity and species composition) with increasing amounts of urban runoff (domestic waste and metals).

Zanella (1982) reported a shift in the relative abundance of two species of caddisflies related to the progressive metals contamination of a California stream. Roline and Boehmke (1981) observed mean diversity of aquatic macroinvertebrates to be lower at metals-polluted sites than non-polluted sites on the same river. Similarly, Kraft and Sypniewski (1981) noted decreases in the average number of macroinvertebrate taxa and number of individuals in samples from a copper polluted area as compared to a non-polluted area of the Keweenaw Waterway, Michigan. The species compositions of macroinvertebrates also was different between the two areas, with chironomids dominant at the copper-polluted sites.

Other than the above, specific documentation of the ecological effects of metals pollution on stream ecosystems is lacking , or so confounded with other stressors, such as

altered pH, temperature, or turbidity, that it is not possible to sort out the effects due solely to metals contamination. Nonetheless, the afore-mentioned studies and the results of laboratory bioassays indicate that the the following effects may be expected as a result of metals contamination: 1) altered fish and benthic invertebrate community structure (e.g. decreased diversity, species composition shifts, loss of intolerant species, establishment of tolerant species); and 2) altered population parameters (e.g. changes in population age structure, decreased reproduction, decreases in growth rate, increased mortality rate, increased incidence of disease, increases in the incidence of spinal deformities and other morphological aberrations).

Two major problems with field biomonitoring studies are the detection of changes in a fluctuating environment, and the assignment of causality to any changes that are noted. The degree of difficulty encountered in the former is directly related to the subtlety of the changes. It is further complicated by the inherent natural variability in population dynamics, compensatory responses of the population to the stressor, and indirect toxic effects (Thomas et al. 1981).

Methodologies for the detection of change under naturally fluctuating conditions have been developed for the assessment of power plant impacts (e.g. Fritz et al. 1980). Two procedures that have been found useful in lessening the problem of natural fluctuations include control-treatment pairing (Skalski and McKenzie 1982), and the use of the ratios of parameters of interest between control and impacted sites, rather than the parameter values themselves (Thomas and Eberhardt 1976). The former utilizes the assumption that if impacted and reference sites are carefully chosen for similarity, then the two stations will track each other, i.e., natural fluctuations are equal at both sites, and thus will cancel each other out. Once control-treatment pairs are established, the use of ratios between the parameter values at "control" and "treatment" sites rather than the parameter values themselves can help to reduce the obfuscating effects of natural fluctuations when analyses are performed through time (e.g. remove the effects of seasonal changes in abundance from the effects of a perturbation at the impacted site). In addition to the expected seasonality in biotic densities and biomasses, significant seasonal variations have been shown for species diversity indices and other biotic indices (Hughes 1978; Murphy 1978).

The establishment of cause-and-effect relationships is even more difficult. Major reasons are the impossibility of random assignment of treatments, and the lack of true replicates. The result is that the influence of other stressors often cannot be evaluated (Thomas et al. 1978). Cause and effect in field studies is perhaps best determined through inference, after eliminating as many alternative explanations as possible, either a priori (by sampling design) or a posteriori (by directly refuting the remaining alternatives).

Goals and Objectives

Given man's seemingly limitless ability to produce toxicants, and an organism's limited ability to protect itself from them, it seems prudent to determine the relationship between environmental contamination and environmental harm. The goal of my research is to determine if presently observed conditions of automotive traffic are causing significant changes in the integrity of roadside stream ecosystems. Recent studies (Van Hassel 1979; Mombeshora et al. 1983) have documented metals contamination of stream ecosystems by automotive traffic. Whether this is a widespread phenomenon is not known. If it is, the

pertinent question then involves determination of the effects of this contamination.

This problem is of no small significance, given the extent of the present highway system and the certainty of its growth. There are also human considerations in that popular fishing locations are often located at highway contact sites. Problems may arise if the habitat becomes unsuitable for game fish, or by the consumption of the contaminated catch.

If it is found that environmental damage can result from highway generated heavy metals, and the damage is deemed to be unacceptable to society, it may be possible for corrective measures in the form of pollution control devices to be installed in the drainage system of highways around streams of particular concern, thereby reducing the contamination. In addition, this information may be taken into consideration during the planning of highway siting, thus preventing the problem in the first place.

To achieve my goal, I sampled heavy metals concentrations and some biotic parameters of fish and benthic invertebrate communities of streams crossed by highways in order to satisfy my three discrete objectives:

1. To determine whether, and, if so, to what degree heavy metals accumulate in various ecosystem components of streams contacted by highways.

2. To assess spatial and temporal differences in fish and benthic invertebrate community level biotic indices of streams contacted by highways.
3. To evaluate the contribution of metals contamination to observed differences in community indices of streams contacted by highways.

METHODS

I investigated the acccumulation of highway-generated heavy metals (zinc, cadmium, and lead) in biotic and abiotic components of six streams crossed by an interstate highway. I also attempted to determine whether metals contamination was significantly altering the fish and macroinvertebrate assemblages of the streams. A sampling protocol was developed that allowed for the assessment of both the spatial aspects of contamination and effects (i.e. upstream of highway vs directly at highway vs downstream of highway), and also the temporal aspects (changes at the same site through time), from shortly after the opening of the highway for a period of 2.5 years.

Study Area

Interstate 295 (I-295) is a recently opened (1980) multi-lane interstate beltline highway just north of Richmond, Virginia. The average daily traffic density increased steadily for the first two years following highway opening (Virginia Department of Highways and Transportation 1981,

1982, 1983, 1984) before levelling off (Table 2). Traffic densities varied somewhat between different segments of I-295, apparently reflecting the preponderance of its use by Richmond commuters, as opposed to motorists travelling from one end of the road to the other. I-295 crosses several small, soft-water, low pH streams along its 36 km generally east to west length through an area ranging from rural farmland to outer suburbs. Six streams were judged to be consistent with the objectives and sampling requirements of this study on the basis of their physical and biological attributes. These small woodlot streams were: Holladay Branch (HO), Beaverdam Creek (BD), Brandy Branch (BR), Sledd's Creek (SL), and two unnamed streams, arbitrarily called Noname (NO) and Natrix Creek (NA). The streams range in average width from 1-3 m, and are all tributaries of the Chickahominy River (Figure 1).

Four of the six streams had three sampling sites on them: an upstream reference site (U), located at least 200 m upstream of the highway; a highway site (H), located directly at the highway (specifically, located at the downstream side of the intersection of the highway with the stream), where contamination should be most pronounced; and a downstream site (D), located 200-500 m downstream from the highway, to assess the extent to which contamination is

TABLE 2

Average Daily Traffic Crossing I-295 Streams

YEAR	STREAM					
	HO	BD	BR	NO	SL	NA
1980	not open	8,015		7,735		
1981	5,370	16,855		10,720		
1982	5,770	17,980		11,880		
1983	6,280	19,050		11,535		

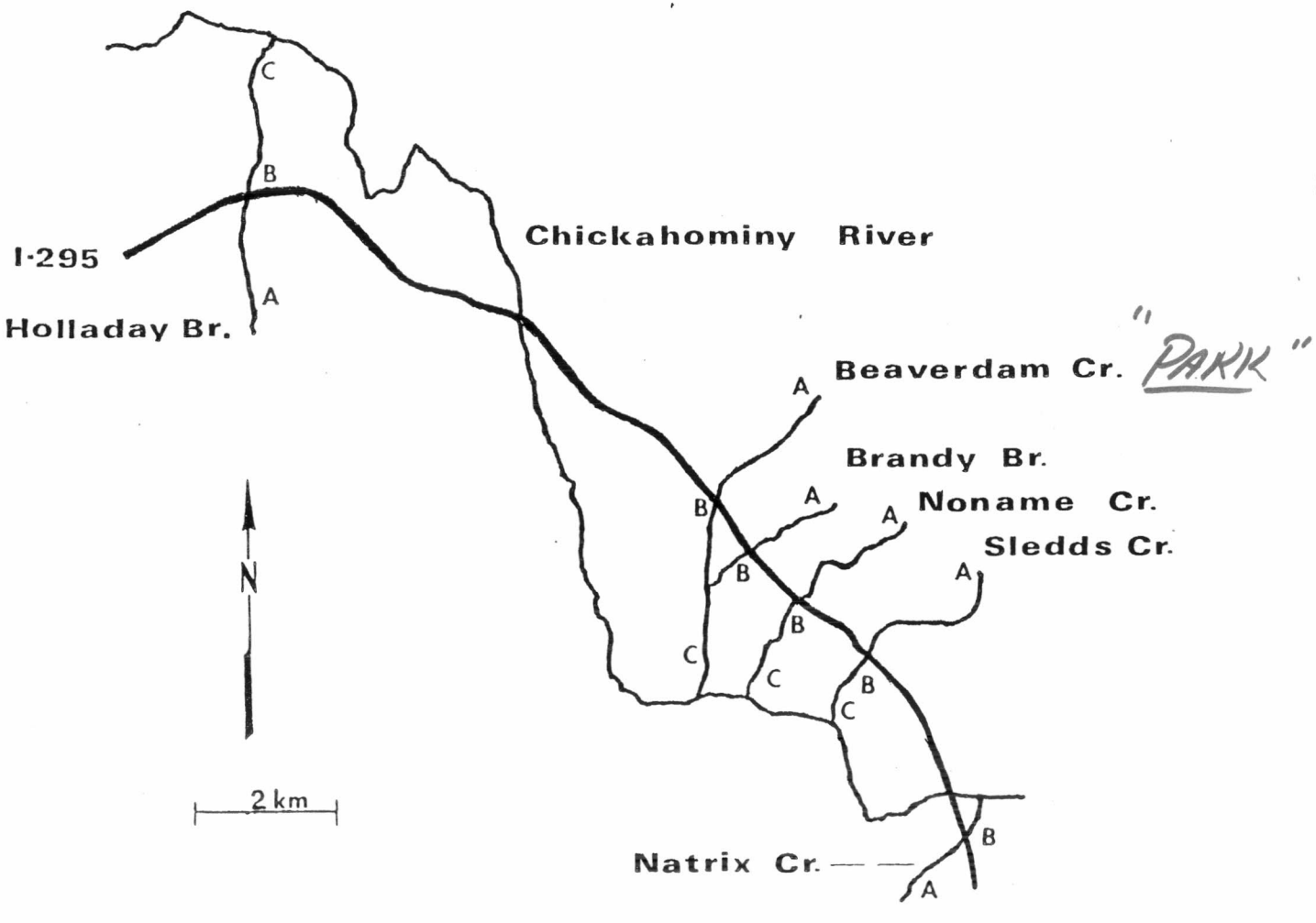


Figure 1. Location of Study Streams.

localized (the spatial component). The upstream site was located at least 200m upstream of the highway to ensure that it would be outside of the zone of contamination. A number of studies, reviewed in Laxen and Harrison (1977) have shown that aerial deposition of lead in the highway environment occurs within a strip 30 m wide on either side of the highway. The tortuous courses of some of the streams, the presence of shoals, and the presence of culverts under I-295 served to restrict fish movement between sites.

Sites on a given stream were selected so as to be as similar to each other as possible, within the constraint of spatial requirements regarding distance from the highway (Table 3). Two of the six streams (Brandy and Natrix) do not have downstream sites because they empty into the Chickahominy River (Natrix), or Beaverdam Creek (Brandy), shortly downstream of the highway site. Water velocities at the highway sites ranged from <0.1 to 0.28 m/s. Velocity increased in the order HO < BD < NA < SL < BR < NO.

The fish assemblages of these streams are characteristic of small low-gradient streams of the region. Cyprinidae was the numerically dominant family. Anguillidae composed a considerable amount of the biomass on many of the streams. Fish assemblages varied somewhat among streams, less among sites on the same stream (Table 4). The number of species

TABLE 3

Physical Characteristics of the I-295 Study Sites[1]

STREAM	SITE	MEAN WIDTH[1]	MEAN DEPTH[1]	MAXIMUM DEPTH[1]	DOMINANT SUBSTRATE
HO	U	0.82	0.10	0.23	sand
	H	0.70	0.35	0.70	silt
	D	1.06	0.20	0.65	sand
BD	U	1.40	0.30	0.65	sand
	H	2.20	0.73	1.05	sand
	D	3.70	0.35	0.75	sand
BR	U	1.70	0.26	0.48	sand
	H	2.00	0.25	0.50	sand
NO	U	1.40	0.17	0.39	sand
	H	1.20	0.27	0.50	sand
	D	1.00	0.15	0.40	sand
SL	U	1.90	0.13	0.27	gravel
	H	1.30	0.16	0.52	gravel
	D	0.73	0.18	0.25	sand
NA	U	2.80	0.40	0.72	pebble
	H	2.40	0.40	0.70	gravel

[1] meters

present per site at any one time ranged from 5-16, and varied from 6-21 over the course of the study. Appendix Table I contains a complete listing of the fish species collected by stream.

Interstate 95 (I-95) is the major north-south interstate highway on the Atlantic seaboard. Traffic densities in the Richmond area average about 50,000 vehicles per day (Virginia Department of Highways and Transportation 1984). I-95 has been in operation in the Richmond area for over 20 years. Several streams crossed by I-95 were sampled once near the end of the study to help assess the effects of long term, high level contamination. Selection factors included proximity to I-295, and similarity to the I-295 streams in both size and general appearance. Care was taken to select sites as similar to each other as availability allowed. Appendix Table II lists the I-95 streams and the types of samples taken at each. Two of these streams also did not have names. I named them MM94.5 Creek and Walthall Creek, after their locations.

The finding of suitable streams along I-95 required more effort than expected. Because of the extensive development along the I-95 "corridor", it was necessary to use a 56 km stretch of the highway, from 16 km north to 40 km south of the intersection of I-95 with I-295 (from mile markers 94 to

TABLE 4

Numerically Dominant Fish Species By Site

STREAM	RANK	UPSTREAM	HIGHWAY	DOWNSTREAM
	1	MM	PP	CC
HO	2	PP	CC	PP
	3	CC	BG	TD
	1	BG	AE	AE
BD	2	AE	BS	LA
	3	PS	CS	CS
	1	BC	TD	
BR	2	CC	LA	--[1]
	3	LA	BC	
	1	BD	BD	LA
NO	2	CC	CC	CC
	3	LA	LA	BD
	1	AE	MM	MM
SL	2	CC	AE	AE
	3	BC	BC	TD
	1	CC	PP	
NA	2	AE	CS	--[1]
	3	CS	AE	

CODE

AE	American eel	CS	Creek chubsucker
BC	Bluehead chub	LA	Least brook lamprey
BD	Blacknose dace	MM	Eastern mudminnow
BG	Bluegill	PP	Pirate perch
BS	Bluespotted sunfish	PS	Pumpkinseed
CC	Creek chub	TD	Tesselated darter

[1] No downstream site.

59). The difficulty was in locating undisturbed-looking streams with the only obvious source of perturbation being the highway at highway sites.

Field Collections

Sampling Schedule

Bimonthly sampling trips to the study area began in March, 1981. On each trip, upstream, highway, and (when present) downstream sites on on two of the six streams were sampled. This was done on a rotating basis. On the first sampling trip, streams BR and NO were sampled, on the second sampling trip streams SL and NA were sampled, and on the third trip streams HO and BD were sampled. On the next trip, sampling returned to streams BR and NO, and so on. Consequently, each stream was sampled twice per year, at six month intervals, for a total of five sampling trips at each site by the time field collections were ended, in July, 1983 (Table 5).

To promote consistency of conditions between trips, the exact dates of the sampling trips were to some extent weather dependent, rather than random or rigidly fixed. The goal was to sample during dry periods with normal water

TABLE 5

Summary of Sampling Schedule

DATE	STREAM					
	HO	BD	BR	NO	SL	NA
MAR 1981			X	X		
MAY 1981					X	X
JULY 1981	X	X				
SEPT 1981			X	X		
NOV 1981					X	X
JAN 1982	X	X				
MAR 1982			X	X		
MAY 1982					X	X
JULY 1982	X	X				
SEPT 1982			X	X		
NOV 1982					X	X
JAN 1983	X	X				
MAR 1983			X	X		
MAY 1983					X	X
JULY 1983	X	X				

levels. The sites on a given stream were sampled in a downstream to upstream order to prevent the activities at one site from possibly affecting conditions at an unsampled site downstream.

Water Quality Sampling

At each site on each trip, water samples were taken and the following parameters determined: water temperature, hardness (mg/l CaCO3), alkalinity (mg/l CaCO3) pH, conductivity (uMhos), and turbidity (% transmitance). This was done to ensure that conditions at the different sites were similar, and secondly to be able to characterize the streams in terms of their susceptibility to metals toxicity.

Metals Sampling

At each site on each trip, the following types and numbers of samples were taken for metals analyses: 1) water (two), total metals; 2) sediment (three), collected from the top three cm of the stream bottom; 3) benthic invertebrates (three per taxon of usually two taxa), collected with a dip net; 4) fish for whole-body analysis (six per species of the most abundant species), collected by electrofishing; and 5) fish for analysis of selected tissues (three each from species large enough for dissection), also taken by

electrofishing. Although larger sample sizes for fish would have been desirable from an analytical standpoint, it was decided that removing a larger number of fish may have caused nontrivial effects on the integrity of the fish community (sensu Falk 1974, 1976).

Water samples for metals analysis were fixed in the field with nitric acid (U.S. Environmental Protection Agency 1974), and all samples were placed in acid-washed polyethylene containers or polyethylene bags, double-bagged, and placed on ice until returned to the lab where they were frozen at -10 C pending analysis.

Biotic Sampling

Concurrent with the sampling for metals, the streams were also sampled in order to provide data to quantitatively describe the fish and benthic macroinvertebrate assemblages. At each site on each trip, a section (ranging from 33-96 m, depending on stream width and fish density) was block-netted and the segment within electrofished by a three man team (three depletion runs) with backpack electroshockers using DC current. The fish collected were anesthetized, identified, counted, weighed to the nearest 0.1 g and measured (total length) to the nearest mm. All fish were then released alive, except for those retained for metals analysis.

Benthic invertebrates were collected for determination of selected community attributes using a 0.26 m^2 circular depletion sampler (Carle 1976). The substrate was stirred for a three minute period, in excess of the time shown to be necesary to obtain a representative sample (Hughes 1978). Three independent samples were taken at each site at depths of 0.2 to 0.4 m. These small Piedmont streams did not have a well developed riffle-pool format. Samples were taken from run-like areas, as similar to each other in substrate as availability allowed. Samples were preserved in formalin pending identification (genus for insects, class for non-insects) and enumeration. Invertebrates were identified using the keys of Merritt and Cummins (1978), Pennak (1978) and Hilsenhoff (1975).

Laboratory Analyses

Metals Determinations

Metals concentrations (ug/g, dry weight) in samples were determined with a Perkin Elmer Model 460 atomic absorption spectrophotometer (AAS), using either standard flame or flameless techniques (Perkin Elmer 1976), depending upon sample mass and expected metals concentrations. Flame spectroscopy was the method of choice due to relative ease of operation and greatly reduced analytical time per sample.

However, extremely small samples, or larger samples with low metals concentrations required the extra sensitivity afforded by the more time-consuming flameless techniques. Flameless AAS work utilized a Perkin Elmer Model HGA 2100 graphite furnace to atomize the sample solutions. Background correction was achieved using a continuous source deuterium lamp. Operational parameters for the devices comprise Appendix Tables III and IV.

The accuracy and precision of metals analyses were determined by: 1) the analysis of bovine liver of known metals concentration (Standard Reference Material 1577, National Bureau of Standards, Washington, D.C.); and 2) the use of spiked samples to determine the percent recovery and to detect matrix interference (U.S. Environmental Protection Agency 1972; Kirchmer 1983). The analysis of reference material gave satisfactory results in all instances (Appendix Tables V and VI). Observed concentrations were within 10% of certified values. The recoveries of spiked samples produced similar results (Appendix Tables VII and VIII). No significant interferences were detected, and recoveries were sufficient to yield accurate determinations (Borg et al. 1981).

Additional quality control was assured by running standard solutions of different concentrations about every

ten samples, and by using procedural blanks at a blank to sample ratio of 1:5 to detect contamination (U.S. Environmental Protection Agency 1972). To minimize sample contamination from extraneous sources, all glassware and polyethylene containers were detergent washed, acid-washed and rinsed in deionized water in a multi-step process, after Martin (1983). After washing, glassware and polyethylene containers were double-bagged in polyethylene bags and stored in a dust-free cabinet until used.

Water.

Fifty ml of the water sample was measured into borosilicate beakers and evaporated to dryness on a hot plate. Three ml of redistilled 16 N nitric acid was then added and the samples were brought to dryness again. Samples were then redissolved to 3 ml with 0.1 N HNO3 and analyzed using flameless techniques, due to low concentrations (U.S. Environmental Protection Agency 1972).

Sediment.

The sediment samples were dried at 60 C, and then sieved through a 500-micron polypropylene screen to ensure uniformity of particle size between samples. This is important because the metals content of sediment samples has been shown to be influenced by grain size, with marked decreases in metals content as grain size increases

(Forstner 1980). One gram of the sieved sample was placed in a polypropylene tube with 8 ml of 5% HNO3 and shaken for 18 h to dissolve into solution any metals (after DiGiulio 1982). The tubes were then centrifuged and the supernatant analyzed using flame (Zn) or flameless (Cd, Pb) methods.

Benthic Invertebrates.

Benthos samples were lyophilized (-50 C, <50 uTorr) for 72 h to reduce variability in weight due to water content, weighed to the nearest 0.0001 g and dry ashed in a muffle furnace at 450 C for at least 12h, to remove organic content. Samples were then redissolved and brought to volume with 0.1 N HNO3. Samples were analyzed using either flame (Zn) or flameless (Cd, Pb) techniques.

Whole-body Fish.

Fish for whole-body analysis were rinsed with deionized water after thawing to remove any debris and excess mucus. Following this, fish were lyophilized (as above), weighed to the nearest 0.0001 g, placed in a beaker, and put into the muffle furnace at 450 C for 12 h. After cooling, two ml of 25% HNO3 was added to the beaker, which was then brought to dryness on a hot plate. Samples were then redissolved and brought to volume with 5% HNO3. Samples were run using flame techniques, since sample masses were always sufficient.

Fish Tissues.

Because certain tissues have special affinities for accumulating different heavy metals, they are often recommended for use in biomonitoring investigations (Simkiss and Taylor 1981; Ney and Van Hassel 1983). In particular, the liver and kidney of fishes have been shown to concentrate heavy metals (Cowx 1982; Paul and Pillai 1983b; Saltes and Bailey 1984). Bone has been shown to accumulate lead, which is structurally similar to calcium, and incorporated in its place (Bowen 1966, 1979). On that basis the analysis of tissues metals was included in this study with the expectation that if any differences in metals concentrations were to be found among the ecosystem components investigated, they would most likely be found in tissues metals.

The liver, kidney and bone (caudal vertebrae) of fish were dissected from partially thawed fish for metals analysis using acid-washed stainless steel and teflon utensils on a clean polyethylene work surface. The use of stainless steel utensils has been shown not to influence metals concentrations in samples (Heit and Klusek 1982). Following dissection, samples were treated in the same manner as benthos samples.

Parameter Estimation

Several biotic parameters were estimated to evaluate different aspects of ecosystem structure. These parameters were selected because they have been shown to, or would intuitively seem to reflect the "health" of the environment and/or changes therein. These parameters were: 1) benthic macroinvertebrate community density (expressed as #/0.26m^2); 2) benthic macroinvertebrate diversity, Shannon Index (Washington 1984); 3) the percentage of the aquatic insect community that was composed of chironomids, AIPC, (Winner et al. 1980); 4) fish species diversity (Shannon Index), computed on the electrofishing sample; 5) fish community density (#/10m), estimated using the maximum weighted likelihood population estimation method of Carle and Strub (1978); and 6) fish community biomass (g/10m), computed by multiplying the population estimate for each species by the average weight of each individual of that species at that site, and then summing those numbers .

Evaluation of changes in these parameters included both differences among upstream, highway, and downstream sites (spatial differences), and differences between these values at the same site through time (temporal differences). Evaluations were further subdivided into general and stream specific components depending on whether all six or just one stream was under consideration.

I also examined the similarity between the structures of the fish community present at the study sites using the coefficient of similarity (B) developed by Pinkham and Pearson (1976). Calculations were made using a computer program developed by the authors. Options used were: 1) group sizes important, 2) 0/0 matches = 1.

$$B = \frac{1}{k} \sum_{i=1}^{k} \frac{\text{MIN } (Xia, Xib)}{\text{MAX } (Xia, Xib)}$$

where k = the number of taxa

and Xia and Xib are the numbers of individuals in the ith taxon for stations a and b

This index takes into account the identity and number of species present, as well as the number of individuals per species, in calculating similarity, and, as a result, in spite of the fact that it has been rarely used in water quality investigations, is considered to be a promising tool (Washington 1984).

The approach used was to compare the fish community present on subsequent trips at each site with the fish

community present the first time the site was sampled. Because seasonal fluctuations in species abundances could obfuscate trends due to metals effects if (for example) spring versus fall comparisons were made, only Trip 1, 3, and 5 at each site were used in this analysis. This restricted comparisons to samples taken at the same time of year.

Accordingly, the fish community at the highway site on Beaverdam Creek (for example) at the start of the study was compared with the community present one and two years later, and a coefficient of similarity computed. This was done for each of the sixteen sites.

Because the similarity index gives no indication of the direction of any changes in fish community structure, it is hypothetically possible that, if the highway sites were in a perturbed condition on the first sampling trip, e.g. from highway construction impact (although none was evident), that any changes noted in fish community structure represented recovery from that impact, and not the effects of highway metals contamination. To address this possibility, I also looked at the similarity between highway and upstream fish community structure on the same time frame as above. If the fish communities were becomming more similar, recovery from an original perturbed condition would

be suspected. If the highway and upstream fish communities became increasingly dissimilar, then impacts due to highway operation would be indicated.

Statistical Procedures

A variety of statistical procedures were employed to establish significance probabilities (p-values) for the various null hypotheses. Due to the characteristically non-normal frequency distributions of metals concentrations in nature (Pinder and Geisy 1981), nonparametric statistical procedures were employed. Statistical significance was assumed at the 0.1 level for Type-I error. Means of median values were used as the index of central tendency for display purposes.

All tests used were one-sided with respect to statistical hypotheses. This was possible because of the a priori information regarding the expected direction of any changes. As a consequence, the p-values associated with respect to the null hypothesis indicated both the direction and magnitude of the difference being tested for, and not just a verdict of significance or not. Even though these tests were one-sided, examination of the p-values makes possible the detection of significance in the non-hypothesized direction, although technically they have no power to do so (but see

below). For example, if the p-value associated with a null hypothesis that states $H \geq U$ is 0.95, then the p-value associated with the alternative hypothesis ($H < U$) is 1 - 0.95 = 0.05. However, since this amounts to making it a two-sided test, the p-value must equal or exceed 0.95 to be significant at an overall alpha level of 0.1. Instances where this occurs are reported.

For reporting purposes the first sampling trip at each of the six streams is reported below as Trip 1, the second as Trip 2, and so on. It should be remembered that the streams sampled contemporaneously are HO and BD, BR and NO, and SL and NA. For each Trip, data from the three sampling trips that constitute one reporting Trip were not pooled in the usual sense (i.e. treated as equivalent, or coming from the same population), but rather statistical procedures were employed that made comparisons only between streams or sites that were directly comparable, before generating the significance probability for each Trip.

There were three basic categories of tests used in the hypothesis testing section of this study. General Spatial Tests addressed the question of whether differences (specifically, location differences) existed in metals levels or biotic parameters between highway sites and upstream reference sites, and separately, between downstream

sites and upstream reference sites on those streams that had downstream sites. Because these tests used data from the different streams, it is their results that are applicible to the question of what are the general, or overall effects or consequences with regard to site type differences. The specific statistical test used to address general spatial differences varied somewhat among the different components of this study due to differences in their respective data bases.

The second general category of tests used in this study was termed General Temporal Tests. These tests addressed the question of whether there were significant monotonic trends (i.e. continually increasing or continually decreasing) in metals levels or biotic parameters at hihgway and, separately, downstream sites, relative to upstream reference sites. These tests were generally performed on ratios constructed by dividing values for highway or downstream sites by the value for the corresponding upstream sites. This was done to eliminate potentially confounding effects (e.g seasonal variations in fish abundance) from obscuring the detection of trends over the five Trips. When, because of the nature of the data, meaningful ratios could not be constructed, general temporal trend testing was performed on the p-values generated by the general spatial tests for each Trip, or on means of median values.

The final category of tests employed in the I-295 hypothesis testing was Stream-specific Temporal Tests. These tests were similar to the general temporal tests in purpose and justification, differing only in the fact that the stream-specific tests were restricted to the analysis of trends on each of the six streams individually. As a consequence of the different data bases (usually six ratios per Trip for the general temporal, and one ratio per Trip for the stream-specific temporal), the statistical tests for stream-specific temporal tests were often different than those for general temporal tests. The specific procedures used in the three general categories for the various parameters are detailed below.

Metals

Water.

General spatial differences in water metals were investigated using a Wilcoxon Signed-Rank test (the nonparametric equivalent of a paired t-test) in which the median metals concentrations of water at highway and downstream sites were paired with concentrations at their corresponding upstream sites. This was done on a per trip basis for each metal, thus for each trip the significance probability associated with the null hypothesis was

generated for each metal. The null hypothesis was that the metals levels at highway or downstream sites was less than or equal to metals levels at upstream sites.

General temporal differences were assessed using Jonckheere's test for ordered alternatives (Jonckheere 1954; Odeh 1971) on the highway/upstream (H/U) and downstream/upstream (D/U) ratios for each Trip. The a priori ordering employed for metal ratios was Trip 1 < Trip 2 < Trip 3 < Trip 4 < Trip 5.

Stream specific temporal changes were assessed using Theil-Sen regression, a nonparametric method of linear regression based on ranks (Hollander and Wolfe 1973). The H/U and D/U ratios for a particular stream were the dependent variables and time (trip) was the independent variable. The null hypothesis was that the slope of the parameter over time was less than or equal to zero. The alternate hypothesis was that the slope was greater than zero (i.e. that metals levels at highway or downstream sites relative to upstream sites increased over time).

Sediment.

General spatial analyses for sediment metals concentrations were conducted using a Wilcoxon Rank Sum test (the nonparametric equivalent of a Student's, or two sample t-test) on the metals concentrations of the three replicates

at highway sites versus the three replicates for the upstream site on the same stream. Downstream versus upstream comparisons were made in the same manner. The six (or in the case of downstream versus upstream comparisons, four) significance probabilities so generated that constituted one Trip were then combined using Fisher's method for combining significance probabilities (Fisher 1970, p. 99) to obtain the global (overall) significance probability for each Trip. This is done by taking the negative of twice the natural logarithm of the individual significance probabilities, noting that the sum of n such numbers has a chi-squared distribution with 2n degrees of freedom under the null hypothesis. The null hypothesis was that highway and separately, downstream sediment metals levels were less than or equal to those found at the upstream sites.

General temporal analyses were made using H/U and D/U ratios constructed from median metals concentrations at each site. These ratios were used in Page's test for ordered alternatives (Page 1963). It was possible to use this test for sediment because (as opposed to water) the data were balanced. This two-way layout allowed for blocking by stream, which results in a more powerful test when differences exist among blocks (streams). The a priori ordering used was that metals ratios increased in the order Trip 1 < Trip 2 < Trip 3 < Trip 4 < Trip 5.

Stream specific temporal analyses were made using Theil-Sen regression on the H/U and D/U ratios for each stream. The null hypothesis was that the slope of the ratios over time was greater than or equal to zero.

Benthic Invertebrates.

Statistical tests for metals concentrations of benthic invertebrates were made more difficult than what would normally be expected due to the nature of the data. The main problems were that varying numbers of benthic invertebrates were collected at each site, and the insects that were collected often differed between sites on the same stream, as well as between streams, and at the same site at different times of the year. Taxon-specific differences have been reported among different classes of benthic invertebrates (Enk and Mathis 1977; Selby et al 1985). Anderson (1977) studied metals concentrations of thirty-five genera of aquatic invertebrates and concluded that with few exceptions (including zinc) that there were no trends toward differences in metals concentrations among the different classes and orders. However, his invertebrates were taken from a variety of locations, both unpolluted and polluted, which would seem to confound the finding of any taxon-specific differences. Consequently, the degree to which the metals concentrations in invertebrates collected in this

study would vary was not known, and was addressed. As a result, it was decided to restrict statistical comparisons to metals concentrations of taxa within the Odonata. Otherwise, statistical testing procedures were the same as those used for sediments.

Whole-body Fish.

The data base for whole body metals concentrations suffered from the same problems as did the benthic invertebrate data base, but to a lesser degree. Sample sizes were generally larger and less variable. That there are species-specific differences in heavy metals content of fishes is more well established (Martin 1983, Van Hassel et al. 1980). In contrast to benthos, the species collected at the different site types on a given stream were usually the same. This made possible a testing by species. However, since the same species were not present at all sites, statistical manipulations were required to assess changes relative to all streams considered collectively, as follows.

General spatial analyses for fish whole bodies were conducted by performing Wilcoxon Rank Sum tests on the metals concentrations of fishes at highway sites versus upstream sites and, separately, for downstream sites versus upstream sites for each species collected on each trip. Specifically, every time three or more of the same species

was collected at both the upstream site and the highway (or downstream) site on a given sampling trip, a Wilcoxon rank sum test was performed to determine whether a significant difference existed between metals concentrations in that species at the two site types, on that trip. This was done for all species collected at that site, thus all possible comparisons of $n \geq 3$ were made.

Doing this resulted in the generation of variable numbers of significance probabilities associated with the null hypotheses that the metals concentrations at highway and downstream sites were less than or equal to metals concentrations at upstream sites. The significance probabilities associated with all of the species on all of the sites that constituted a given Trip were then combined using Fisher's method to obtain the global significance probability associated with the null hypothesis for each Trip.

General temporal analyses for fish whole-body metals concentrations were performed using Theil-Sen regression on the p-values established by general spatial analyses for each Trip. This was done instead of ratios (as above) because no meaningful ratio could be constructed. The null hypothesis was that the slope of the significance probabilities over time was greater than or equal to zero.

The alternate hypothesis stated that through time there was a trend for the significance probabilities to decrease (i.e. the sites became more different).

Stream-specific temporal analyses were performed in the same manner as general temporal tests, using only the significance probabilities for each stream.

Fish Tissues.

The data base for fish tissues was similar in some respects to both the benthos data base, and the fish whole-body data base. Species differences were known to exist, and there was no one species that was present at all sites. The statistical tests that were used reflected the differing similarities to the two other data bases.

General spatial analyses were made for tissues metals in the same manner as for whole-body fish. The tissue-metal combinations examined were liver: zinc, cadmium, lead; kidney: zinc, cadmium, lead; bone: zinc, lead.

General temporal changes were assessed in the same manner as used for benthos: using Page's test for ordered alternatives. Because no one species was present or collected at every site on every trip, and the resulting imbalance, the test was performed on H/U and D/U ratios constructed from the median metals concentration of all fish at a site pooled. Although species-specific differences in

metals concentrations exist, a chi-square test for independence showed that the distributions of the various species was independent of site type (i.e. U, H, or D). This reduces possible problems of bias due to different species being consistently present at different sites. The a priori ordering used was that the ratio for Trip 1 < Trip 2 < Trip 3 < Trip 4 < Trip 5.

Biotic Parameters

General Spatial Tests.

The general spatial conundrum for the biotic parameters was addressed using Wilcoxon signed-rank tests in which parameter values for highway and downstream sites were paired with those for their corresponding upstream sites. This was done on a per trip basis, the result being that for each Trip a significance probability associated with the null hypothesis was generated for each biotic parameter. The null hypothesis was that the value of the biotic parameter (e.g. density, biomass, diversity) at highway or downstream sites was greater than or equal to that for the upstream sites. The only exception to this was in the case of the percentage of the aquatic insect community that was composed of chironomids (AIPC), in which instance the inequality was reversed, because unlike the other parameters, the

percentage of chironomids was expected to increase with increasing metals.

General Temporal Tests.

General temporal changes were evaluated using Page's test for ordered alternatives. This two-way layout allowed for blocking by stream, which increases the power of the test when there are differences between streams. The test was made on the highway/upstream and downstream/upstream ratios. The a priori ordering used was Trip 1 > Trip 2 > Trip 3 > Trip 4 > Trip 5 for all parameters except AIPC in which case the ordering was reversed, for the same reason as before.

Stream-Specific Spatial Tests.

Stream-specific temporal analyses were made using Theil-Sen regression. The highway/upstream and downstream/upstream ratios for a particular stream were the dependent variables and time (trip) was the independent variable. The null hypotheses for each parameter was that the slope of the parameter over time was greater than or equal to zero. The alternate hypotheses were that the slope was less than zero (i.e. that the parameters decreased). Again, the inequalities were reversed in the case of AIPC.

Relationship Between Metals and Biotic Parameters

This section differs from the foregoing in that while the former utilized statistical hypothesis testing or "inductive" hypothesis testing (Quinn and Dunham 1983) in order to identify differences between site types or changes at sites through time, within the context of a field "experiment", the present is more descriptive or phenomonological (Strong 1980). Here I attempt to identify relationships between metals levels and biotic parameters by examining how they co-vary over their ranges at the 80 site-trip combinations. Differences between the two approaches will be apparent if the models employed in the hypothesis testing section are not fully correct. In these instances the descriptive approach will be more enlightening because rather than evaluating biotic parameters against upstream-ness, highway-ness, Trip 1-ness, etc. the biotic parameters at a site are evaluated directly against existing metals concentrations at that site.

The approach I used was to first construct scatter plots, in which the median sediment metals concentration for a site on a given trip was plotted against the value of the biotic parameter for that site, on that trip. This was done for all metals and all biotic parameters. These plots were inspected for suggestions of linear dependency

(correlations), and threshold-like effects. These would be suspected when the biotic parameter appeared to be independent of metals concentrations, until a certain threshold concentration was reached. Above this threshold, the parameter would appear not to be independent of metals concentrations.

The main statistical tool in these evaluations was Spearman Rank Correlation, which tests the degree of linear association (dependency) between two random variables. Each of the sediment metals (zinc, cadmium, and lead) were evaluated against all of the biotic parameters. The null hypotheses were that there was no correlation between sediment metals levels and biotic parameters.

If a threshold-like response was suggested by the graph, a Wilcoxon rank sum test was performed to test whether statistically significant differences existed between the values of the biotic parameters above and below threshold metals concentrations.

The median sediment metals concentrations for a site on each trip was used as the index of metals contamination. Sediment metals were chosen over the other possibile indicators of contamination because sediment was present at every site (unlike fish of a given species, or benthos), and there were no missing samples (as in the case of water). In

addition, Forstner (1980), and Feltz (1980) conclude that sediment metals analyses are preferable to water metals analyses in assessing the degree of metals contamination at a site because water borne concentrations often fluctuate greatly over short time intervals, and are more difficult from an analytical standpoint. In this study, water was also deemed inappropriate due to smaller sample size (n=2 versus n=3) and the expectations concerning the sporadic nature of its contamination. Sediment metals levels were evaluated against benthic invertebrate density, diversity, AIPC, fish density, fish diversity, and fish biomass.

RESULTS

A total of 10,700 metals determinations were made with respect to the I-295 hypothesis testing for spatial and temporal differences. Pursuant to the determination of biotic parameters for benthic invertebrates, over 75,000 invertebrates were identified and enumerated. Similarly, 10,000 fish were collected identified, measured, and weighed over the fifteen sampling trips. Approximately 1000 statistical tests were performed in the compilation of the results, which follow.

Water Quality

Average water quality parameters for the study period suggested no differences in water quality (except for conductivity) among upstream, highway, and downstream sites on any stream (Table 6). Although there were small differences in hardness, alkalinity, conductivity, and turbidity among the different streams, overall they were very similar: soft-water and of mildly acidic to neutral pH. Conductivity was generally higher at the highway and downstream sites, and was more variable. Conductivity ranged

from 14-120, 18-190, and 59-95 uMhos at upstream, highway, and downstream sites respectively. Streams HO and NO had the highest conductivities.

Metals Contamination

Water

Metals levels in water were consistently low, in the parts per billion range. There were differences in concentrations among the metals, with zinc levels being the highest, lead intermediate, and cadmium lowest. Although metals levels often tended to be higher at highway sites, the differences were, for the most part, not statistically significant. The inadvertent loss of some water samples resulted in missing values for nine of the 80 site-trip combinations.

Zinc.

Median water-borne zinc concentrations ranged from 0.002 to 0.142 mg/l. The mean of the median values for each stream and trip showed no major or consistent differences among upstream, highway, or downstream sites (Table 7). An abnormally high concentration of zinc (0.142 mg/l) was found for stream BD on Trip 5. The H/U and D/U ratios suggest no major trends through time (Table 8).

TABLE 6

Mean Water Quality Parameters over the Study Period

STREAM	SITE	Hardness[1]	Alkalinity[1]	pH	Conductivity[2]	Turbidity[3]
	U	20.6	19.3	6.85	74.2	0.86
HO	H	34.0	33.0	6.85	112.6	0.87
	D	33.5	25.7	6.68	112.2	0.84
	U	18.5	14.3	7.28	59.4	0.88
BD	H	21.7	15.3	7.38	75.8	0.94
	D	25.2	15.5	6.86	80.0	0.93
	U	22.2	11.0	6.65	78.8	0.97
BR	H	18.2	11.6	6.78	81.4	0.97
	U	18.0	11.5	6.70	47.8	0.94
NO	H	29.8	13.8	6.43	114.6	0.90
	D	29.6	13.4	6.68	116.3	0.93
	U	28.0	26.2	6.98	88.8	0.93
SL	H	30.2	24.6	6.85	75.4	0.91
	D	29.4	25.6	6.93	88.4	0.93
	U	16.2	20.6	6.95	69.8	0.93
NA	H	15.8	16.6	7.06	66.8	0.91
	U	20.6	17.2	6.90	69.8	0.92
MEAN	H	25.0	19.2	6.89	87.8	0.92
	D	29.4	20.1	6.79	99.2	0.91

[1] mg/l as CaCO3
[2] uMhos
[3] % transmittance

TABLE 7

Median Zinc Levels (mg/l) by Site and Trip for Water

STREAM	SITE	TRIP				
		1	2	3	4	5
HO	U	0.016	0.018	0.023	0.023	0.028
	H	0.014	0.013	0.010	0.012	0.021
	D	0.012	-	0.027	0.023	0.019
BD	U	0.009	0.010	0.009	0.010	0.023
	H	0.012	0.007	0.066	0.009	0.028
	D	0.012	0.006	0.010	0.008	0.142
BR	U	-	-	-	0.008	0.011
	H	0.009	-	-	0.009	0.014
NO	U	0.009	-	0.011	0.009	0.008
	H	0.006	0.009	0.005	0.014	0.012
	D	0.009	0.008	0.013	0.002	0.010
SL	U	0.007	-	0.009	0.048	0.011
	H	0.011	-	0.020	0.015	0.013
	D	0.012	0.017	0.012	0.010	0.011
NA	U	0.011	0.012	0.016	0.014	0.015
	H	0.012	0.021	0.040	0.020	0.014
MEAN	U	0.010	0.013	0.014	0.019	0.016
	H	0.011	0.012	0.028	0.013	0.017
	D	0.011	0.010	0.016	0.011	0.046

TABLE 8

Water Zinc Temporal Ratios

STREAM		TRIP 1	2	3	4	5
HO	H/U	0.863	0.713	0.425	0.511	0.750
	D/U	0.716	-	1.174	0.979	0.696
BD	H/U	1.401	0.679	7.506	0.857	1.217
	D/U	1.343	0.597	1.091	0.762	6.196
BR	H/U	-	-	-	1.146	1.318
NO	H/U	0.656	-	0.474	1.637	1.562
	D/U	1.000	-	1.256	0.222	1.250
SL	H/U	1.642	-	2.063	0.305	1.227
	D/U	1.836	-	1.311	0.201	1.000
NA	H/U	1.137	1.776	2.422	1.442	0.967
MEAN	H/U	1.140	1.056	2.578	0.983	1.174
	D/U	1.224	0.597	1.208	0.541	2.286

Statistical analyses revealed no significant differences in zinc concentrations between site types (general spatial) or at the same site through time (both the general and the stream-specific temporal). The only exception was that zinc concentrations at downstream sites on Trip 3 were higher than those at upstream sites (Table 9).

Cadmium.

Median cadmium concentrations in water for the study sites ranged from <0.001 to 0.639 ug/l. Mean values for upstream, highway, and downstream sites for each trip suggested no major or consistent differences between site types (Table 10). Inspection of the H/U and D/U cadmium ratios also indicated no major differences over the course of the study (Table 11). These ratios were more variable and in most cases larger (or smaller) than those found for zinc, but this is a mathematical artifact due to the small size of the denominator, in relation to the sensitivity of the analytical procedure.

There were no statistically significant differences in cadmium concentrations between site types on the different trips, or in the H/U or D/U ratios through time (Table 12). The one exception was that cadmium concentrations were

TABLE 9

Water Zinc Significance Probabilities

I. General Spatial (Wilcoxon Signed-Rank)

	TRIP				
	1	2	3	4	5
H ≤ U	.312	.625	.219	.656	.281
D ≤ U	.375	-	.062	.938	.375

II. General Temporal (Jonckheere's Test)

H/U	D/U
.510	.765

III. Stream Specific Temporal (Theil-Sen)

	STREAM					
	HO	BD	BR	NO	SL	NA
H/U	.592	.592	-	-	-	.592
D/U	-	.408	-	-	-	-

TABLE 10

Median Cadmium Concentrations (ug/l) by Trip for Water

		TRIP				
STREAM	SITE	1	2	3	4	5
	U	0.039	0.052	0.049	0.049	0.443
HO	H	0.029	0.025	0.013	0.002	0.521
	D	0.013	-	0.088	0.032	0.293
	U	0.051	0.029	0.007	0.010	0.235
BD	H	0.071	0.005	0.336	0.004	0.639
	D	0.035	0.009	0.076	0.001	0.114
	U	-	-	-	0.040	0.039
BR	H	<0.001	-	-	0.050	0.051
	U	0.001	-	0.071	0.010	<0.001
NO	H	0.019	<0.001	0.031	0.060	0.034
	D	0.007	0.005	0.088	0.003	0.037
	U	0.004	-	0.203	0.535	0.021
SL	H	0.005	-	0.083	0.098	0.032
	D	0.016	0.107	0.187	0.065	0.017
	U	0.013	0.017	0.275	0.059	0.030
NA	H	0.005	0.037	0.343	0.141	0.036
	U	0.022	0.033	0.121	0.117	0.128
MEAN	H	0.022	0.017	0.161	0.059	0.219
	D	0.018	0.040	0.110	0.025	0.115

TABLE 11

Water Cadmium Metals Ratios

STREAM		TRIP 1	2	3	4	5
HO	H/U	0.756	0.490	0.263	0.040	1.176
	D/U	0.333	-	1.788	0.646	0.663
BD	H/U	1.402	0.169	44.800	0.450	2.715
	D/U	0.696	0.305	10.200	0.150	0.484
BR	H/U	-	-	-	1.263	1.321
NO	H/U	19.500	-	0.441	6.050	68.000
	D/U	7.000	-	1.238	0.300	75.000
SL	H/U	1.250	-	0.409	0.183	1.548
	D/U	4.000	-	0.924	0.122	0.810
NA	H/U	0.370	2.143	1.247	2.398	1.217
MEAN	H/U	4.656	0.934	9.432	1.731	12.663
	D/U	3.007	0.305	3.537	0.305	19.239

significantly higher at highway sites on Trip 5 than at upstream sites. On this Trip the mean value for highway sites was 0.219 mg/l as opposed to 0.128 mg/l for upstream sites.

Lead.

Water lead concentrations for the study sites ranged from 0.185 to 4.895 ug/l. Mean values for the different site types suggested no major or consistent differences between upstream, highway, or downstream sites over the course of the study (Table 13). Inspection of the H/U and D/U ratios revealed no pattern for H/U or D/U changes (Table 14).

The statistical analyses revealed no significant differences among site types on any trip (Table 15). Jonckheere's test indicated a significant increasing trend in the H/U lead ratios. This means that lead levels progressively increased at highway sites relative to upstream sites over the course of the study. No stream-specific trends were found. However, because of the lost samples, only four (of the usual ten) tests could be made.

TABLE 12

Water Cadmium Significance Probabilities

I. General Spatial (Wilcoxon Signed-Rank)

	TRIP				
	1	2	3	4	5
H ≤ U	.312	.750	.500	.500	.016
D ≤ U	.688	-	.125	.938	.812

II. General Temporal (Jonckheere's Test)

H/U	D/U
.201	.778

III. Stream Specific Temporal (Theil-Sen)

	STREAM					
	HO	BD	BR	NO	SL	NA
H/U	.592	.408	-	-	-	.408
D/U	-	.592	-	-	-	-

TABLE 13

Median Lead Concentrations (ug/l) by Trip for Water

STREAM	SITE	TRIP 1	2	3	4	5
	U	2.430	0.925	3.955	1.915	1.380
HO	H	2.215	1.000	1.150	0.635	2.285
	D	1.305	-	3.121	1.680	0.860
	U	1.080	1.400	1.822	1.230	2.140
BD	H	0.685	0.185	3.490	0.330	1.395
	D	1.030	0.285	1.680	0.255	1.325
	U	-	-	-	0.205	0.715
BR	H	0.230	-	-	0.800	0.725
	U	0.700	-	0.720	0.435	0.410
NO	H	0.310	0.640	0.320	0.815	0.730
	D	1.130	0.296	0.380	0.200	0.490
	U	1.040	-	1.055	4.895	0.620
SL	H	0.230	-	0.405	1.472	1.290
	D	0.650	1.690	0.323	0.626	0.450
	U	1.235	0.446	1.950	1.223	1.340
NA	H	0.590	1.750	3.090	1.653	1.535
	U	1.297	0.924	1.900	1.650	1.101
MEAN	H	0.710	0.894	1.691	0.951	1.327
	D	1.029	0.757	1.376	0.690	0.781

TABLE 14

Water Lead Metals Ratios

STREAM		TRIP 1	2	3	4	5
HO	H/U	0.912	1.081	0.291	0.332	1.656
	D/U	0.537	-	0.789	0.877	0.623
BD	H/U	0.634	0.132	1.915	0.268	0.652
	D/U	0.954	0.204	0.922	0.207	0.619
BR	H/U	-	-	-	3.902	1.014
NO	H/U	0.443	-	0.444	1.874	1.780
	D/U	1.614	-	0.528	0.460	1.195
SL	H/U	0.221	-	0.384	0.301	2.081
	D/U	0.625	-	0.306	0.128	0.726
NA	H/U	0.478	3.924	1.585	1.352	1.146
MEAN	H/U	0.538	1.712	0.924	1.338	1.388
	D/U	0.933	0.204	0.636	0.418	0.791

TABLE 15

Water Lead Significance Probabilities

I. General Spatial (Wilcoxon Signed-Rank)

	TRIP				
	1	2	3	4	5
H ≤ U	.969	.375	.500	.781	.156
D ≤ U	.688	-	.938	.938	.875

II. General Temporal (Jonckheere's Test)

H/U	D/U
.065	.664

III. Stream Specific Temporal (Theil-Sen)

	STREAM					
	HO	BD	BR	NO	SL	NA
H/U	.408	.408	-	-	-	.592
D/U	-	.408	-	-	-	-

Sediment

Sediment metals levels varied widely among streams, among sites on the same stream, and at some sites through time. In general, metals levels at highway sites were significantly higher than those at upstream sites, while those at downstream sites were not. Temporally, sediment metals levels seemed to rise and then plateau, rather than continually increase. The concentrations of the different metals were correlated with each other ($p=0.001$, Spearman's $r=0.72$ 0.66, and 0.75 for Zn-Cd, Zn-Pb, and Cd-Pb, respectively. Thus, when the concentration of one metal was high at a site, the concentrations of the other metals were usually high also.

Zinc.

Major differences were found in sediment zinc concentrations between site types and at highway sites over time. Zinc concentrations ranged from 0.86 to 116.45 ug/g. Mean values for the streams by Trip showed zinc levels to average two to six times higher at highway sites than at upstream or downstream sites (Table 16). There was much inter-stream variation in the extent to which zinc levels increased at highway sites. Two streams (HO,NA) showed

major, two intermediate (BD,BR), and two minor, if any, differences between upstream and highway sites (NO,SL).

The sediment zinc H/U and D/U ratios further substantiate the above observations (Table 17). They also indicate that zinc concentrations at the highway did not steadily increase relative to upstream sites, but rather peaked at Trip 3 and then levelled off, or perhaps even declined.

The statistical tests showed that zinc concentrations were significantly higher at highway sites than at upstream reference sites on Trips 1, 3, and 4, and higher downstream than upstream on Trip 1 (Table 18). Page's test for general temporal changes showed no significant increasing trend in the ratios over the course of the study. The same lack of trends was noted in the stream-specific tests.

Cadmium.

Median sediment cadmium values for the sites over the course of the study ranged from 0.005 to 0.770 ug/g. Mean values for the different site types showed that cadmium concentrations at highway sites averaged 1.2 to 10 times higher than at upstream sites on each Trip (Table 19). Downstream cadmium levels were higher on some trips. Again, there was much inter-stream variability in the extent to which the different highway sites became contaminated. Highway sites on streams HO and NA showed the greatest

TABLE 16

Median Sediment Zinc Levels (ug/g) by Site and Trip

STREAM	SITE	TRIP 1	2	3	4	5
HO	U	2.01	1.67	1.49	2.32	6.71
	H	16.03	33.00	47.84	52.78	43.01
	D	10.98	16.84	20.19	16.18	5.09
BD	U	2.64	8.19	7.38	7.01	4.07
	H	17.66	4.50	14.57	9.45	2.55
	D	12.34	4.63	3.54	5.76	5.45
BR	U	2.52	14.40	1.49	2.54	2.00
	H	2.04	10.07	1.80	1.49	1.68
NO	U	2.56	3.99	1.26	0.86	1.99
	H	1.89	2.97	2.40	1.54	1.84
	D	5.20	2.20	2.55	1.84	1.52
SL	U	19.77	5.68	11.50	4.36	8.16
	H	11.88	7.14	5.60	5.35	5.11
	D	9.65	5.36	6.69	5.09	8.33
NA	U	20.12	17.28	6.60	5.87	4.73
	H	59.84	83.49	116.45	48.12	40.46
MEAN	U	8.27	8.54	4.95	3.83	4.61
	H	18.22	23.53	31.44	19.79	15.77
	D	9.54	7.26	8.24	7.22	5.10

TABLE 17

Sediment Zinc Ratios

STREAM		TRIP 1	2	3	4	5
HO	H/U	7.976	19.761	32.107	22.752	6.409
	D/U	5.463	10.084	13.550	6.976	0.758
BD	H/U	6.700	0.549	1.974	1.348	0.628
	D/U	4.683	0.565	0.480	0.822	1.340
BR	H/U	0.810	0.700	1.208	0.587	0.839
NO	H/U	0.738	0.744	1.905	1.791	0.922
	D/U	2.031	0.551	2.024	2.140	0.764
SL	H/U	0.601	1.257	0.487	1.227	0.626
	D/U	0.488	0.945	0.582	1.167	1.020
NA	H/U	2.974	4.831	17.644	8.198	8.554
MEAN	H/U	3.300	4.640	9.221	5.984	2.996
	D/U	3.166	3.036	4.159	2.776	0.971

TABLE 18

Sediment Zinc Significance Probabilities

I. General Spatial (Wilcoxon Rank Sum)

	TRIP				
	1	2	3	4	5
H ≤ U	.02	.50	.01	.06	.75
D ≤ U	.05	.37	.14	.24	.76

II. General Temporal (Page's Test)

H/U	D/U
.207	.345

III. Stream Specific Temporal (Theil-Sen)

	STREAM					
	HO	BD	BR	NO	SL	NA
H/U	.500	.758	.500	.242	.500	.117
D/U	.592	.500	-	.500	.117	-

contamination. The H/U ratios were greater than unity on all trips, and D/U ratios greater than unity on four of five trips (Table 20). The pattern for cadmium ratios paralleled that of the zinc ratios.

Sediment cadmium levels were significantly higher at highway sites than upstream sites on trips 1, 2, 3, and 4. Downstream sites were not significantly higher than upstream sites on any Trip (Table 21). General temporal analyses showed no significant continually increasing trend over time for either the H/U or D/U ratios. Stream specific analyses likewise indicated no such trend on any stream.

Lead.

Median sediment lead levels for the different sites over the course of the study ranged from 0.76 to 33.40 ug/g. Mean values for all streams combined suggested that highway sites had consistently higher lead levels than either upstream or downstream sites (Table 22). The general pattern of inter-stream variability noted for zinc and cadmium was also apparent for lead. Streams NA and HO had the highest lead concentrations. Sediment lead ratios also indicated consistently higher lead levels at highway sites with respect to upstream sites, with the ratio peaking on Trip 3 (Table 23).

TABLE 19

Median Sediment Cadmium Levels (ug/g) by Site and Trip

		TRIP				
STREAM	SITE	1	2	3	4	5
	U	0.011	0.012	0.006	0.008	0.022
HO	H	0.031	0.135	0.310	0.226	0.184
	D	0.045	0.067	0.022	0.084	0.020
	U	0.008	0.014	0.009	0.018	0.016
BD	H	0.066	0.034	0.055	0.036	0.006
	D	0.054	0.035	0.015	0.031	0.023
	U	0.012	0.065	0.011	0.024	0.008
BR	H	0.010	0.040	0.010	0.014	0.008
	U	0.018	0.018	0.015	0.006	0.010
NO	H	0.011	0.008	0.023	0.007	0.005
	D	0.017	0.006	0.028	0.006	0.004
	U	0.022	0.011	0.019	0.008	0.024
SL	H	0.008	0.007	0.025	0.014	0.004
	D	0.010	0.006	0.009	0.008	0.012
	U	0.064	0.023	0.046	0.039	0.026
NA	H	0.086	0.410	0.770	0.250	0.298
	U	0.022	0.024	0.018	0.017	0.018
MEAN	H	0.035	0.106	0.199	0.091	0.084
	D	0.031	0.028	0.018	0.032	0.015

TABLE 20

Sediment Cadmium Ratios

STREAM		TRIP 1	2	3	4	5
HO	H/U	2.818	11.250	51.667	28.250	8.264
	D/U	4.091	5.583	3.667	10.500	0.909
BD	H/U	8.250	2.429	6.111	2.000	0.375
	D/U	6.750	2.500	1.667	1.722	1.437
BR	H/U	0.833	0.615	0.909	0.583	1.000
NO	H/U	0.611	0.444	1.533	1.167	0.500
	D/U	0.944	0.333	1.867	1.000	0.400
SL	H/U	0.364	0.636	1.316	1.750	0.167
	D/U	0.455	0.545	0.474	1.000	0.500
NA	H/U	1.344	17.826	16.739	6.410	11.462
MEAN	H/U	2.370	5.534	13.046	6.693	3.645
	D/U	3.060	2.241	1.918	3.556	0.812

TABLE 21

Sediment Cadmium Significance Probabilities

I. General Spatial (Wilcoxon Rank Sum)

	TRIP				
	1	2	3	4	5
H ≤ U	.08	.08	.03	.01	.24
D ≤ U	.13	.18	.15	.40	.79

II. General Temporal (Page's Test)

H/U	D/U
.533	.726

III. Stream Specific Temporal (Theil-Sen)

	STREAM					
	HO	BD	BR	NO	SL	NA
H/U	.408	.958	.408	.500	.408	.500
D/U	.592	.958	-	.500	.242	-

TABLE 22

Median Sediment Lead Levels (ug/g) by Site and Trip

STREAM	SITE	TRIP 1	2	3	4	5
HO	U	4.63	1.60	1.90	1.54	6.99
	H	13.77	28.90	31.00	27.65	24.42
	D	6.83	13.30	9.80	2.27	2.30
BD	U	6.10	3.30	2.20	3.08	4.25
	H	16.40	2.50	7.20	6.21	1.83
	D	10.49	2.40	2.80	2.70	4.26
BR	U	4.43	9.50	2.50	2.30	1.93
	H	3.50	6.40	3.10	1.70	3.40
NO	U	3.90	4.60	1.60	1.40	1.92
	H	2.10	3.10	2.80	1.70	0.76
	D	1.40	0.80	1.60	1.30	1.31
SL	U	4.80	2.90	3.10	2.00	2.70
	H	0.80	1.80	4.10	2.60	1.28
	D	1.80	1.50	1.20	1.30	2.00
NA	U	10.46	4.63	5.90	19.40	4.33
	H	15.54	20.10	33.40	14.00	14.15
MEAN	U	5.72	4.42	2.87	4.95	3.69
	H	8.69	10.47	13.60	8.98	7.64
	D	5.13	4.50	3.85	1.90	2.47

Statistical testing revealed significantly higher lead levels at highway sites compared to upstream sites on Trips 1, 3, and 4 (Table 24). There were no statistically significant differences between upstream and downstream sediment lead levels. No general temporal trends were significant. A stream-specific increasing trend was noted for stream SL, however the ratio never exceeded unity, indicating that highway lead levels increased relative to, but never surpassed, upstream lead levels.

Benthic Invertebrates

Heavy metals concentrations in benthic invertebrates varied for the different taxa, with median metals concentrations for the different species varying two to four fold (Table 25). The most extreme concentrations were found for tipulids and trichopterans. Because the remaining insects were all odonates and more similar in metals concentrations, I deleted the tipulids and trichopterans from further consideration and pooled the odonates together for the following analyses.

Zinc.

TABLE 23

Sediment Lead Ratios

STREAM		TRIP 1	2	3	4	5
HO	H/U	2.973	18.063	16.316	18.000	3.496
	D/U	1.475	8.313	5.158	1.481	0.329
BD	H/U	2.689	0.758	3.273	2.017	0.431
	D/U	1.720	0.727	1.273	0.878	1.001
BR	H/U	0.790	0.674	1.240	0.739	1.763
NO	H/U	0.538	0.764	1.750	1.214	0.395
	D/U	0.359	0.174	1.000	0.929	0.684
SL	H/U	0.167	0.621	1.323	1.300	0.473
	D/U	0.375	0.517	0.387	0.650	0.739
NA	H/U	1.486	4.346	5.661	0.722	3.268
MEAN	H/U	1.440	4.189	4.927	3.999	1.637
	D/U	0.982	2.433	1.954	0.984	0.688

TABLE 24

Sediment Lead Significance Probabilities

I. General Spatial (Wilcoxon Rank Sum)

	TRIP				
	1	2	3	4	5
H ≤ U	.02	.28	.03	.04	.14
D ≤ U	.12	.53	.23	.67	.76

II. General Temporal (Page's Test)

H/U	D/U
.435	.242

III. Stream Specific Temporal (Theil-Sen)

	STREAM					
	HO	BD	BR	NO	SL	NA
H/U	.500	.758	.242	.500	.408	.500
D/U	.758	.592	-	.408	.042	-

TABLE 25

Median Metals Levels for Benthic Invertebrates

Taxa	n	Zn	Cd	Pb
Diptera				
Tipulidae	60	75.8	0.38	4.59
Odonata				
Coenagrionidae	19	102.6	0.41	2.17
Libellulidae	22	115.6	0.31	3.24
Gomphidae	103	117.4	0.28	1.95
Aeshnidae	124	126.9	0.50	1.67
Macromiidae	17	131.1	0.62	2.47
Calopterygidae	96	135.6	0.64	2.23
Cordulegastridae	80	158.9	0.44	2.76
Trichoptera				
Hydropsychidae	19	184.9	0.32	1.20

Median zinc concentrations in odonates from the study sites ranged from 95.8 to 168.3 ug/g. Mean values across streams reveal no differences in odonate zinc concentrations among the different site types (Table 26). Mean H/U and D/U ratios vary very slightly around unity (Table 27). Stream-specific ratios indicate inconsistent differences between site types.

Statistical analyses revealed significantly higher zinc concentrations in odonates at highway sites on Trip 4 and at downstream sites on Trips 1, 3, and 4 (Table 28). There was no trend towards continually increasing metals levels in either a general or stream-specific context.

Cadmium.

Odonate cadmium concentrations ranged from 0.12 to 10.28 ug/g. Mean values over streams by trip show elevated cadmium levels at highway sites on Trips 2 and 3 (Table 29), rising from 0.47 (Trip 1) to 2.26 ug/g (Trip 3). Odonate cadmium ratios indicate elevated cadmium levels at highway and, to a lesser extent, downstream sites after Trip 1 (Table 30). Cadmium concentrations on odonates from streams BR and NA seemed to be higher than those found in the other streams.

TABLE 26

Median Zinc Concentrations (ug/g) for Odonates

STREAM	SITE	TRIP 1	2	3	4	5
	U	148.3	163.7	130.6	124.1	154.3
HO	H	118.9	157.2	109.9	110.2	82.1
	D	125.1	132.3	116.7	130.4	132.9
	U	99.6	112.1	115.0	96.1	97.0
BD	H	127.8	111.3	130.6	118.4	111.6
	D	150.7	128.1	156.4	119.9	121.9
	U	151.4	122.2	138.5	146.8	151.4
BR	H	137.6	148.4	167.9	133.2	137.6
	U	159.3	165.2	161.3	147.6	166.5
NO	H	105.2	140.4	119.9	115.0	140.2
	D	159.2	152.2	153.7	123.5	147.3
	U	112.0	95.8	114.8	141.6	134.7
SL	H	122.5	103.4	112.2	-	131.9
	D	110.5	-	124.2	168.3	121.4
	U	118.5	149.8	112.9	142.4	121.2
NA	H	139.1	149.6	131.6	153.2	121.9
	U	131.5	134.8	128.8	133.1	137.5
MEAN	H	125.2	135.0	128.7	126.0	120.9
	D	136.4	137.5	137.7	135.6	130.9

TABLE 27

Odonate Zinc Ratios

STREAM		TRIP 1	2	3	4	5
HO	H/U	0.802	0.960	0.842	0.888	0.532
	D/U	0.843	0.808	0.893	1.051	0.862
BD	H/U	1.283	0.993	1.135	1.232	1.151
	D/U	1.512	1.142	1.359	1.248	1.257
BR	H/U	0.909	1.214	1.212	0.907	0.909
NO	H/U	0.661	0.850	0.743	0.779	0.842
	D/U	1.000	0.922	0.953	0.837	0.885
SL	H/U	1.094	1.079	0.977	-	0.979
	D/U	0.987	-	1.082	1.189	0.901
NA	H/U	1.174	0.999	1.166	1.076	1.006
MEAN	H/U	0.987	1.016	1.013	0.976	0.903
	D/U	1.085	0.957	1.072	1.081	0.976

TABLE 28

Odonate Zinc Significance Probabilities

I. General Spatial (Wilcoxon Rank Sum)

	TRIP				
	1	2	3	4	5
H ≤ U	.13	.37	.69	.08	.79
D ≤ U	.05	.39	.04	.10	.27

II. General Temporal (Page's Test)

H/U	D/U
.764	.718

III. Stream Specific Temporal (Theil-Sen)

	STREAM					
	HO	BD	BR	NO	SL	NA
H/U	.592	.500	.675	.242	-	.758
D/U	.242	.592	-	.883	-	-

TABLE 29

Median Cadmium Concentrations (ug/g) for Odonates

STREAM	SITE	TRIP 1	2	3	4	5
	U	0.44	0.28	0.39	0.26	0.36
HO	H	0.25	0.24	0.48	0.23	0.33
	D	0.14	0.22	0.23	0.31	0.49
	U	0.36	0.24	0.16	0.23	0.36
BD	H	0.51	0.32	1.15	0.39	0.27
	D	0.76	0.58	0.82	0.41	0.71
	U	1.15	0.50	0.84	0.52	2.35
BR	H	0.63	0.72	10.28	0.68	0.54
	U	0.44	0.26	0.52	0.38	0.85
NO	H	0.12	7.61	0.59	0.31	0.38
	D	0.26	0.32	0.35	0.74	0.51
	U	0.18	0.27	0.46	0.36	0.27
SL	H	0.14	0.13	0.25	-	1.10
	D	0.12	-	0.22	0.27	0.57
	U	0.68	1.21	1.19	1.33	0.61
NA	H	1.16	0.37	0.81	0.66	0.67
	U	0.54	0.46	0.53	0.51	0.80
MEAN	H	0.47	1.57	2.26	0.46	0.55
	D	0.32	0.37	0.40	0.43	0.58

Statistical analyses revealed that odonate cadmium levels were significantly higher than upstream reference levels at highway sites on Trips 2 and 3, and at downstream sites on Trips 2, 4, and 5 (Table 31). Although no significant trends were found in the general temporal sense, a significant increasing trend was found for the D/U ratio on stream HO.

Lead.

Odonate lead concentrations ranged from 0.38 to 11.28 ug/g. Mean values across streams suggest no major or consistent differences among site types (Table 32). If anything, odonate lead levels appear higher at upstream sites. However, mean odonate lead concentrations at highway sites were lowest on Trip 1 and averaged about twice that concentration on subsequent trips. Mean odonate cadmium concentrations at downstream sites increased rather steadily over the course of the study. The odonate lead ratios showed no consistent differences by trip (Table 33).

Statistical analyses revealed that odonate lead concentrations were significantly higher at highway sites on Trip 2 (Table 34). Suprisingly, they were significantly higher at upstream sites than highway sites on Trip 1.

TABLE 30

Odonate Cadmium Ratios

STREAM		TRIP 1	2	3	4	5
HO	H/U	0.573	0.859	1.253	0.866	0.917
	D/U	0.311	0.777	0.581	1.189	1.361
BD	H/U	1.395	1.361	7.108	1.740	0.750
	D/U	2.085	2.415	5.059	1.806	1.972
BR	H/U	0.551	1.446	12.305	1.324	0.229
NO	H/U	0.266	29.613	1.126	0.831	0.447
	D/U	0.599	1.249	0.673	1.968	0.606
SL	H/U	0.741	0.491	0.549	-	4.074
	D/U	0.657	-	0.487	0.759	2.111
NA	H/U	1.709	0.308	0.680	0.497	1.098
MEAN	H/U	0.872	5.680	3.834	1.052	1.253
	D/U	0.913	1.481	1.700	1.430	1.513

TABLE 31

Odonate Cadmium Significance Probabilities

I. General Spatial (Wilcoxon Rank Sum)

	TRIP				
	1	2	3	4	5
H ≤ U	.70	.03	.03	.14	.57
D ≤ U	.13	.02	.34	.05	.04

II. General Temporal (Page's Test)

H/U	D/U
.464	.209

III. Stream Specific Temporal (Theil-Sen)

	STREAM					
	HO	BD	BR	NO	SL	NA
H/U	.117	.592	.592	.592	-	.500
D/U	.042	.592	-	.408	-	-

TABLE 32

Median Lead Concentrations (ug/g) for Odonates

STREAM	SITE	TRIP 1	2	3	4	5
HO	U	2.76	5.09	5.74	2.90	4.44
	H	1.87	2.65	4.63	2.13	6.21
	D	1.72	1.24	2.02	4.75	4.59
BD	U	2.74	1.31	2.33	2.26	1.62
	H	1.39	1.57	1.35	1.88	0.88
	D	2.87	1.52	2.33	2.31	3.01
BR	U	3.77	2.30	4.43	1.16	2.13
	H	1.57	1.94	4.37	1.03	3.03
NO	U	1.30	2.80	2.78	2.15	3.95
	H	1.41	11.28	1.83	1.05	2.37
	D	1.95	2.10	2.17	1.37	3.46
SL	U	0.55	0.84	1.02	1.44	1.80
	H	0.38	1.95	0.80	-	1.97
	D	0.58	-	1.03	1.33	1.09
NA	U	2.36	3.66	3.10	4.24	4.88
	H	1.90	2.88	4.63	4.53	4.73
MEAN	U	2.25	2.67	3.23	2.36	3.14
	H	1.42	3.71	2.94	2.13	3.20
	D	1.78	1.62	1.89	2.44	3.04

General temporal analyses showed a significant increasing trend in the H/U ratios over the course of the study. In other words, there was a significant trend towards increasing lead levels at highway sites in relation to upstream sites. Stream specific analyses indicated a significant increasing trend in the H/U ratio for stream BR.

Whole-body Fish

The analysis of whole-body metals concentrations was, like benthic invertebrate metals analysis, complicated by the fact that fish metals burdens are to some extent species specific (Martin 1983; Ney and Van Hassel 1983). This is especially true for zinc which is under at least partial homeostatic control (Bryan 1976). Different species from the same site often had significantly different metals concentrations. Unfortunately, no one species was present at every site on every trip. Although the statistical procedures employed were selected to be immune to this problem, the presentation of median metals values was not. Nonetheless, the mean of median fish whole body metals values across streams by site type are presented for reference, as to the range of concentrations encountered

TABLE 33

Odonate Lead Ratios

STREAM		TRIP 1	2	3	4	5
HO	H/U	0.678	0.521	0.807	0.735	1.399
	D/U	0.623	0.244	0.352	1.639	1.033
BD	H/U	0.508	1.198	0.579	0.834	0.541
	D/U	1.047	1.160	1.000	1.023	1.853
BR	H/U	0.416	0.843	0.985	0.884	1.422
NO	H/U	1.080	4.029	0.658	0.488	0.600
	D/U	1.494	0.752	0.781	0.637	0.877
SL	H/U	0.691	2.321	0.789	-	1.094
	D/U	1.055	-	1.015	0.924	0.606
NA	H/U	0.803	0.788	1.495	1.067	0.968
MEAN	H/U	0.696	1.617	0.886	0.802	1.004
	D/U	1.055	0.719	0.787	1.056	1.092

TABLE 34

Odonate Lead Significance Probabilities

I. General Spatial (Wilcoxon Rank Sum)

	TRIP				
	1	2	3	4	5
H ≤ U	.98	.08	.92	.94	.23
D ≤ U	.85	.77	.88	.85	.58

II. General Temporal (Page's Test)

H/U	D/U
.090	.282

III. Stream Specific Temporal (Theil-Sen)

	STREAM					
	HO	BD	BR	NO	SL	NA
H/U	.117	.500	.042	.883	-	.408
D/U	.242	.408	-	.592	-	-

(Table 35). As expected, there was quite a range in median zinc concentrations by species, less for cadmium and lead (Table 36).

As regards site type differences, the mean values for the pooled fishes show no major or consistent differences for zinc. Cadmium and lead levels appear to be higher at upstream sites than at highway or downstream sites. Mean cadmium and lead levels at highway sites appear to be increasing over the course of the study. However, the same is true for Cd and Pb at upstream sites, and for Cd at downstream sites.

Zinc.

General spatial statistical analysis revealed significantly higher fish whole body zinc concentrations at highway sites on Trips 4 and 5, and at downstream sites on Trips 2, 4, and 5 (Table 37), compared to upstream sites.

General temporal analysis showed a significant decreasing trend in the significance probabilities associated with the highway versus upstream general spatial tests for each trip. In other words, there was a significant decreasing trend in the probability that zinc levels in fish at highway sites was less than or equal to that at upstream sites. Stream

TABLE 35

Mean Fish Whole-body Metal Burdens (ug/g) by Site Type

METAL	SITE	TRIP 1	2	3	4	5
ZINC	U	91.7	119.0	115.0	111.4	113.9
	H	67.5	118.2	103.0	101.6	101.6
	D	82.2	139.7	123.9	126.4	107.9
CADMIUM	U	0.128	0.168	0.195	0.150	0.254
	H	0.071	0.108	0.148	0.145	0.177
	D	0.103	0.113	0.187	0.141	0.319
LEAD	U	1.08	2.17	1.29	1.34	1.86
	H	0.74	0.89	0.99	1.10	1.06
	D	0.94	0.85	0.68	0.64	1.06

TABLE 36

Median Fish Whole-Body Metals Burdens (ug/g) by Species

Species	n	Zn	Cd	Pb
Least brook lamprey	183	81.0	0.050	0.70
American eel	273	88.4	0.170	0.80
Pirate perch	91	91.6	0.190	1.17
Creek chubsucker	98	98.9	0.090	0.60
Tesselated darter	59	106.0	0.230	0.90
Bluegill	66	116.1	0.069	0.50
Bluehead chub	93	127.6	0.180	0.50
Creek chub	226	136.3	0.130	0.63
Blacknose dace	80	207.1	0.157	1.00
Eastern mudminnow	17	289.6	0.181	1.12

TABLE 37

Fish Whole-body Zinc Significance Probabilities

I. General Spatial (Wilcoxon Rank Sum; Fisher's)

	TRIP				
	1	2	3	4	5
H ≤ U	.72	.11	.52	.002	.001
D ≤ U	.77	.06	.19	.004	.009

II. General Temporal (Page's Test)

H/U	D/U
.042	.117

III. Stream Specific Temporal (Theil-Sen)

	STREAM					
	HO	BD	BR	NO	SL	NA
H/U	.117	.958	.500	.167	.042	.833
D/U	.167	.500	-	.500	.408	-

specific analyses indicated a significant decreasing trend in the p-values for the highway vs upstream pairing for stream SL.

Cadmium.

General spatial statistical analysis revealed significantly higher whole body cadmium concentrations at highway sites on Trip 4 and at downstream sites on Trips 2, 3, 4, and 5 (Table 38), compared to upstream sites. The general temporal analysis showed a significant decreasing trend in the significance probabilities associated with the downstream vs upstream comparison. There was no such trend for the H/U ratio. Stream specific analyses indicated significant decreasing trends in the H vs U p-values for stream SL and in the D vs U p-values for streams HO and SL.

Lead.

General spatial statistical analysis revealed that whole fish lead levels were not significantly higher at highway or downstream sites on any Trip (Table 39). In fact, lead levels were significantly higher at upstream sites than highway or downstream sites on Trips 2, 3, and 4.

General temporal analysis showed no trend towards decreasing p-values over the course of the study. Stream specific temporal analyses did show a significant trend towards decreasing p-values (i.e. increasing lead) at the

TABLE 38

Fish Whole-body Cadmium Significance Probabilities

I. General Spatial (Wilcoxon Rank Sum; Fisher's)

	TRIP				
	1	2	3	4	5
H ≤ U	.35	.80	.84	.001	.18
D ≤ U	.50	.075	.001	.001	.001

II. General Temporal (Page's Test)

H/U	D/U
.408	.080

III. Stream Specific Temporal (Theil-Sen)

	STREAM					
	HO	BD	BR	NO	SL	NA
H/U	.408	.592	.500	.833	.042	.438
D/U	.042	.167	-	.167	.008	-

TABLE 39

Fish Whole-body Lead Significance Probabilities

I. General Spatial (Wilcoxon Rank Sum; Fisher's)

	TRIP				
	1	2	3	4	5
H ≤ U	.63	.95	.99	.99	.85
D ≤ U	.80	.97	.97	.99	.46

II. General Temporal (Page's Test)

H/U	D/U
.675	.546

III. Stream Specific Temporal (Theil-Sen)

	STREAM					
	HO	BD	BR	NO	SL	NA
H/U	.500	.117	.625	.042	.180	.958
D/U	.500	.375	-	.500	.820	-

highway site for stream NO. Conversely, it also showed a trend towards increasing p-values (decreasing lead with respect to upstream) for stream NA.

Fish Tissues

Metals levels in fish tissues were subject to the same interspecies variations that plagued fish whole-bodies. Again, statistical tests were used that were immune to these potentially confounding effects. An additional problem encountered was a wide variation in metals levels in the livers of the various cyprinids. This was most likely due to the diffuse nature of the liver in these fish, and the extreme difficulty in extracting the entire organ, which is nonhomogeneous with respect to metals levels (Heit 1979).

American eels were the most numerous of the fish used for tissues analysis. Their organs were discrete and easily dissectable in toto. Their metals levels were less variable and are presented for reference (Table 40).

Liver Zinc.

General spatial statistical analysis revealed no significantly higher liver zinc concentrations at highway or downstream sites than at upstream sites (Table 41). In fact, liver zinc levels were significantly lower at highway sites than upstream sites on Trip 3. General temporal

TABLE 40

Median Eel Tissues Metals Levels (ug/g) by Site and Trip

		TRIP				
TISSUE	SITE	1	2	3	4	5
Liver Zn	U	--	145.4	167.2	134.7	150.1
	H	140.6	132.5	131.7	117.6	141.1
	D	142.1	114.6	122.7	141.4	132.4
Liver Cd	U	--	10.68	3.84	2.79	5.42
	H	2.04	4.49	3.05	2.29	4.23
	D	6.03	3.86	2.76	3.68	5.54
Liver Pb	U	-	2.61	2.90	4.13	4.61
	H	1.46	1.95	2.12	1.19	1.01
	D	3.60	3.20	1.07	1.12	0.87
Kidney Zn	U	-	204.4	146.0	146.4	117.3
	H	108.1	173.8	134.6	137.2	122.5
	D	133.7	157.7	146.5	157.8	100.2
Kidney Cd	U	-	21.04	9.71	6.59	8.32
	H	6.69	8.72	6.12	5.69	4.33
	D	3.50	10.32	9.05	7.24	3.00
Kidney Pb	U	-	7.98	5.28	4.73	2.95
	H	1.60	3.15	2.95	2.04	1.74
	D	2.62	9.31	2.00	1.07	0.81
Bone Zn	U	-	74.9	68.6	82.7	71.1
	H	61.6	73.3	77.5	71.1	81.6
	D	69.2	66.7	77.2	83.4	56.2
Bone Pb	U	-	7.31	7.74	8.21	9.86
	H	3.53	5.07	4.81	2.80	3.73
	D	10.33	11.47	2.67	3.28	4.58

analysis showed no trends towards increasingly higher liver zinc at highway or downstream sites over time relative to upstream sites.

Liver Cadmium.

General spatial statistical analysis revealed significantly higher liver cadmium levels at downstream sites than upstream sites on Trips 3 and 4 (Table 42). General temporal analysis showed no trends toward increasing cadmium levels at either highway or downstream sites, relative to upstream sites.

Liver Lead.

General spatial statistical analysis revealed no significantly higher liver lead levels at highway or downstream sites compared with upstream sites (Table 43). General temporal analysis showed no trend towards increasing liver lead levels at highway or downstream sites relative to upstream.

Kidney Zinc.

General spatial statistical analysis revealed that kidney zinc concentrations at highway and downstream sites were not significantly higher than those from fish at upstream sites (Table 44). General temporal analysis showed no trend towards increasing kidney zinc levels at highway or downstream sites relative to upstream.

TABLE 41

Liver Zinc Significance Probabilities

I. General Spatial (Wilcoxon Rank Sum; Fisher's)

	TRIP				
	1	2	3	4	5
H ≤ U	.63	.69	.96	.53	.82
D ≤ U	.80	.85	.75	.70	.91

II. General Temporal (Theil-Sen)

H/U	D/U
.592	.500

TABLE 17

Sediment Zinc Ratios

STREAM		TRIP 1	2	3	4	5
HO	H/U	7.976	19.761	32.107	22.752	6.409
	D/U	5.463	10.084	13.550	6.976	0.758
BD	H/U	6.700	0.549	1.974	1.348	0.628
	D/U	4.683	0.565	0.480	0.822	1.340
BR	H/U	0.810	0.700	1.208	0.587	0.839
NO	H/U	0.738	0.744	1.905	1.791	0.922
	D/U	2.031	0.551	2.024	2.140	0.764
SL	H/U	0.601	1.257	0.487	1.227	0.626
	D/U	0.488	0.945	0.582	1.167	1.020
NA	H/U	2.974	4.831	17.644	8.198	8.554
MEAN	H/U	3.300	4.640	9.221	5.984	2.996
	D/U	3.166	3.036	4.159	2.776	0.971

TABLE 18

Sediment Zinc Significance Probabilities

I. General Spatial (Wilcoxon Rank Sum)

	TRIP				
	1	2	3	4	5
H ≤ U	.02	.50	.01	.06	.75
D ≤ U	.05	.37	.14	.24	.76

II. General Temporal (Page's Test)

H/U	D/U
.207	.345

III. Stream Specific Temporal (Theil-Sen)

	STREAM					
	HO	BD	BR	NO	SL	NA
H/U	.500	.758	.500	.242	.500	.117
D/U	.592	.500	-	.500	.117	-

contamination. The H/U ratios were greater than unity on all trips, and D/U ratios greater than unity on four of five trips (Table 20). The pattern for cadmium ratios paralleled that of the zinc ratios.

Sediment cadmium levels were significantly higher at highway sites than upstream sites on trips 1, 2, 3, and 4. Downstream sites were not significantly higher than upstream sites on any Trip (Table 21). General temporal analyses showed no significant continually increasing trend over time for either the H/U or D/U ratios. Stream specific analyses likewise indicated no such trend on any stream.

Lead.

Median sediment lead levels for the different sites over the course of the study ranged from 0.76 to 33.40 ug/g. Mean values for all streams combined suggested that highway sites had consistently higher lead levels than either upstream or downstream sites (Table 22). The general pattern of inter-stream variability noted for zinc and cadmium was also apparent for lead. Streams NA and HO had the highest lead concentrations. Sediment lead ratios also indicated consistently higher lead levels at highway sites with respect to upstream sites, with the ratio peaking on Trip 3 (Table 23).

TABLE 19

Median Sediment Cadmium Levels (ug/g) by Site and Trip

		TRIP				
STREAM	SITE	1	2	3	4	5
	U	0.011	0.012	0.006	0.008	0.022
HO	H	0.031	0.135	0.310	0.226	0.184
	D	0.045	0.067	0.022	0.084	0.020
	U	0.008	0.014	0.009	0.018	0.016
BD	H	0.066	0.034	0.055	0.036	0.006
	D	0.054	0.035	0.015	0.031	0.023
	U	0.012	0.065	0.011	0.024	0.008
BR	H	0.010	0.040	0.010	0.014	0.008
	U	0.018	0.018	0.015	0.006	0.010
NO	H	0.011	0.008	0.023	0.007	0.005
	D	0.017	0.006	0.028	0.006	0.004
	U	0.022	0.011	0.019	0.008	0.024
SL	H	0.008	0.007	0.025	0.014	0.004
	D	0.010	0.006	0.009	0.008	0.012
	U	0.064	0.023	0.046	0.039	0.026
NA	H	0.086	0.410	0.770	0.250	0.298
	U	0.022	0.024	0.018	0.017	0.018
MEAN	H	0.035	0.106	0.199	0.091	0.084
	D	0.031	0.028	0.018	0.032	0.015

TABLE 20

Sediment Cadmium Ratios

STREAM		TRIP 1	2	3	4	5
HO	H/U	2.818	11.250	51.667	28.250	8.264
	D/U	4.091	5.583	3.667	10.500	0.909
BD	H/U	8.250	2.429	6.111	2.000	0.375
	D/U	6.750	2.500	1.667	1.722	1.437
BR	H/U	0.833	0.615	0.909	0.583	1.000
NO	H/U	0.611	0.444	1.533	1.167	0.500
	D/U	0.944	0.333	1.867	1.000	0.400
SL	H/U	0.364	0.636	1.316	1.750	0.167
	D/U	0.455	0.545	0.474	1.000	0.500
NA	H/U	1.344	17.826	16.739	6.410	11.462
MEAN	H/U	2.370	5.534	13.046	6.693	3.645
	D/U	3.060	2.241	1.918	3.556	0.812

TABLE 21

Sediment Cadmium Significance Probabilities

I. General Spatial (Wilcoxon Rank Sum)

	TRIP				
	1	2	3	4	5
H ≤ U	.08	.08	.03	.01	.24
D ≤ U	.13	.18	.15	.40	.79

II. General Temporal (Page's Test)

H/U	D/U
.533	.726

III. Stream Specific Temporal (Theil-Sen)

	STREAM					
	HO	BD	BR	NO	SL	NA
H/U	.408	.958	.408	.500	.408	.500
D/U	.592	.958	-	.500	.242	-

TABLE 22

Median Sediment Lead Levels (ug/g) by Site and Trip

STREAM	SITE	TRIP 1	2	3	4	5
HO	U	4.63	1.60	1.90	1.54	6.99
	H	13.77	28.90	31.00	27.65	24.42
	D	6.83	13.30	9.80	2.27	2.30
BD	U	6.10	3.30	2.20	3.08	4.25
	H	16.40	2.50	7.20	6.21	1.83
	D	10.49	2.40	2.80	2.70	4.26
BR	U	4.43	9.50	2.50	2.30	1.93
	H	3.50	6.40	3.10	1.70	3.40
NO	U	3.90	4.60	1.60	1.40	1.92
	H	2.10	3.10	2.80	1.70	0.76
	D	1.40	0.80	1.60	1.30	1.31
SL	U	4.80	2.90	3.10	2.00	2.70
	H	0.80	1.80	4.10	2.60	1.28
	D	1.80	1.50	1.20	1.30	2.00
NA	U	10.46	4.63	5.90	19.40	4.33
	H	15.54	20.10	33.40	14.00	14.15
MEAN	U	5.72	4.42	2.87	4.95	3.69
	H	8.69	10.47	13.60	8.98	7.64
	D	5.13	4.50	3.85	1.90	2.47

Statistical testing revealed significantly higher lead levels at highway sites compared to upstream sites on Trips 1, 3, and 4 (Table 24). There were no statistically significant differences between upstream and downstream sediment lead levels. No general temporal trends were significant. A stream-specific increasing trend was noted for stream SL, however the ratio never exceeded unity, indicating that highway lead levels increased relative to, but never surpassed, upstream lead levels.

Benthic Invertebrates

Heavy metals concentrations in benthic invertebrates varied for the different taxa, with median metals concentrations for the different species varying two to four fold (Table 25). The most extreme concentrations were found for tipulids and trichopterans. Because the remaining insects were all odonates and more similar in metals concentrations, I deleted the tipulids and trichopterans from further consideration and pooled the odonates together for the following analyses.

Zinc.

TABLE 23

Sediment Lead Ratios

STREAM		TRIP 1	2	3	4	5
HO	H/U	2.973	18.063	16.316	18.000	3.496
	D/U	1.475	8.313	5.158	1.481	0.329
BD	H/U	2.689	0.758	3.273	2.017	0.431
	D/U	1.720	0.727	1.273	0.878	1.001
BR	H/U	0.790	0.674	1.240	0.739	1.763
NO	H/U	0.538	0.764	1.750	1.214	0.395
	D/U	0.359	0.174	1.000	0.929	0.684
SL	H/U	0.167	0.621	1.323	1.300	0.473
	D/U	0.375	0.517	0.387	0.650	0.739
NA	H/U	1.486	4.346	5.661	0.722	3.268
MEAN	H/U	1.440	4.189	4.927	3.999	1.637
	D/U	0.982	2.433	1.954	0.984	0.688

TABLE 24

Sediment Lead Significance Probabilities

I. General Spatial (Wilcoxon Rank Sum)

	TRIP				
	1	2	3	4	5
H ≤ U	.02	.28	.03	.04	.14
D ≤ U	.12	.53	.23	.67	.76

II. General Temporal (Page's Test)

H/U	D/U
.435	.242

III. Stream Specific Temporal (Theil-Sen)

	STREAM					
	HO	BD	BR	NO	SL	NA
H/U	.500	.758	.242	.500	.408	.500
D/U	.758	.592	-	.408	.042	-

TABLE 25

Median Metals Levels for Benthic Invertebrates

Taxa	n	Zn	Cd	Pb
Diptera				
Tipulidae	60	75.8	0.38	4.59
Odonata				
Coenagrionidae	19	102.6	0.41	2.17
Libellulidae	22	115.6	0.31	3.24
Gomphidae	103	117.4	0.28	1.95
Aeshnidae	124	126.9	0.50	1.67
Macromiidae	17	131.1	0.62	2.47
Calopterygidae	96	135.6	0.64	2.23
Cordulegastridae	80	158.9	0.44	2.76
Trichoptera				
Hydropsychidae	19	184.9	0.32	1.20

Median zinc concentrations in odonates from the study sites ranged from 95.8 to 168.3 ug/g. Mean values across streams reveal no differences in odonate zinc concentrations among the different site types (Table 26). Mean H/U and D/U ratios vary very slightly around unity (Table 27). Stream-specific ratios indicate inconsistent differences between site types.

Statistical analyses revealed significantly higher zinc concentrations in odonates at highway sites on Trip 4 and at downstream sites on Trips 1, 3, and 4 (Table 28). There was no trend towards continually increasing metals levels in either a general or stream-specific context.

Cadmium.

Odonate cadmium concentrations ranged from 0.12 to 10.28 ug/g. Mean values over streams by trip show elevated cadmium levels at highway sites on Trips 2 and 3 (Table 29), rising from 0.47 (Trip 1) to 2.26 ug/g (Trip 3). Odonate cadmium ratios indicate elevated cadmium levels at highway and, to a lesser extent, downstream sites after Trip 1 (Table 30). Cadmium concentrations on odonates from streams BR and NA seemed to be higher than those found in the other streams.

TABLE 26

Median Zinc Concentrations (ug/g) for Odonates

STREAM	SITE	TRIP 1	2	3	4	5
HO	U	148.3	163.7	130.6	124.1	154.3
	H	118.9	157.2	109.9	110.2	82.1
	D	125.1	132.3	116.7	130.4	132.9
BD	U	99.6	112.1	115.0	96.1	97.0
	H	127.8	111.3	130.6	118.4	111.6
	D	150.7	128.1	156.4	119.9	121.9
BR	U	151.4	122.2	138.5	146.8	151.4
	H	137.6	148.4	167.9	133.2	137.6
NO	U	159.3	165.2	161.3	147.6	166.5
	H	105.2	140.4	119.9	115.0	140.2
	D	159.2	152.2	153.7	123.5	147.3
SL	U	112.0	95.8	114.8	141.6	134.7
	H	122.5	103.4	112.2	-	131.9
	D	110.5	-	124.2	168.3	121.4
NA	U	118.5	149.8	112.9	142.4	121.2
	H	139.1	149.6	131.6	153.2	121.9
MEAN	U	131.5	134.8	128.8	133.1	137.5
	H	125.2	135.0	128.7	126.0	120.9
	D	136.4	137.5	137.7	135.6	130.9

TABLE 27

Odonate Zinc Ratios

STREAM		TRIP 1	2	3	4	5
HO	H/U	0.802	0.960	0.842	0.888	0.532
	D/U	0.843	0.808	0.893	1.051	0.862
BD	H/U	1.283	0.993	1.135	1.232	1.151
	D/U	1.512	1.142	1.359	1.248	1.257
BR	H/U	0.909	1.214	1.212	0.907	0.909
NO	H/U	0.661	0.850	0.743	0.779	0.842
	D/U	1.000	0.922	0.953	0.837	0.885
SL	H/U	1.094	1.079	0.977	-	0.979
	D/U	0.987	-	1.082	1.189	0.901
NA	H/U	1.174	0.999	1.166	1.076	1.006
MEAN	H/U	0.987	1.016	1.013	0.976	0.903
	D/U	1.085	0.957	1.072	1.081	0.976

TABLE 28

Odonate Zinc Significance Probabilities

I. General Spatial (Wilcoxon Rank Sum)

	TRIP				
	1	2	3	4	5
H ≤ U	.13	.37	.69	.08	.79
D ≤ U	.05	.39	.04	.10	.27

II. General Temporal (Page's Test)

H/U	D/U
.764	.718

III. Stream Specific Temporal (Theil-Sen)

	STREAM					
	HO	BD	BR	NO	SL	NA
H/U	.592	.500	.675	.242	-	.758
D/U	.242	.592	-	.883	-	-

TABLE 29

Median Cadmium Concentrations (ug/g) for Odonates

		TRIP				
STREAM	SITE	1	2	3	4	5
	U	0.44	0.28	0.39	0.26	0.36
HO	H	0.25	0.24	0.48	0.23	0.33
	D	0.14	0.22	0.23	0.31	0.49
	U	0.36	0.24	0.16	0.23	0.36
BD	H	0.51	0.32	1.15	0.39	0.27
	D	0.76	0.58	0.82	0.41	0.71
	U	1.15	0.50	0.84	0.52	2.35
BR	H	0.63	0.72	10.28	0.68	0.54
	U	0.44	0.26	0.52	0.38	0.85
NO	H	0.12	7.61	0.59	0.31	0.38
	D	0.26	0.32	0.35	0.74	0.51
	U	0.18	0.27	0.46	0.36	0.27
SL	H	0.14	0.13	0.25	-	1.10
	D	0.12	-	0.22	0.27	0.57
	U	0.68	1.21	1.19	1.33	0.61
NA	H	1.16	0.37	0.81	0.66	0.67
	U	0.54	0.46	0.53	0.51	0.80
MEAN	H	0.47	1.57	2.26	0.46	0.55
	D	0.32	0.37	0.40	0.43	0.58

Statistical analyses revealed that odonate cadmium levels were significantly higher than upstream reference levels at highway sites on Trips 2 and 3, and at downstream sites on Trips 2, 4, and 5 (Table 31). Although no significant trends were found in the general temporal sense, a significant increasing trend was found for the D/U ratio on stream HO.

Lead.

Odonate lead concentrations ranged from 0.38 to 11.28 ug/g. Mean values across streams suggest no major or consistent differences among site types (Table 32). If anything, odonate lead levels appear higher at upstream sites. However, mean odonate lead concentrations at highway sites were lowest on Trip 1 and averaged about twice that concentration on subsequent trips. Mean odonate cadmium concentrations at downstream sites increased rather steadily over the course of the study. The odonate lead ratios showed no consistent differences by trip (Table 33).

Statistical analyses revealed that odonate lead concentrations were significantly higher at highway sites on Trip 2 (Table 34). Suprisingly, they were significantly higher at upstream sites than highway sites on Trip 1.

TABLE 30

Odonate Cadmium Ratios

STREAM		TRIP 1	2	3	4	5
HO	H/U	0.573	0.859	1.253	0.866	0.917
	D/U	0.311	0.777	0.581	1.189	1.361
BD	H/U	1.395	1.361	7.108	1.740	0.750
	D/U	2.085	2.415	5.059	1.806	1.972
BR	H/U	0.551	1.446	12.305	1.324	0.229
NO	H/U	0.266	29.613	1.126	0.831	0.447
	D/U	0.599	1.249	0.673	1.968	0.606
SL	H/U	0.741	0.491	0.549	-	4.074
	D/U	0.657	-	0.487	0.759	2.111
NA	H/U	1.709	0.308	0.680	0.497	1.098
MEAN	H/U	0.872	5.680	3.834	1.052	1.253
	D/U	0.913	1.481	1.700	1.430	1.513

TABLE 31

Odonate Cadmium Significance Probabilities

I. General Spatial (Wilcoxon Rank Sum)

	TRIP				
	1	2	3	4	5
H ≤ U	.70	.03	.03	.14	.57
D ≤ U	.13	.02	.34	.05	.04

II. General Temporal (Page's Test)

H/U	D/U
.464	.209

III. Stream Specific Temporal (Theil-Sen)

	STREAM					
	HO	BD	BR	NO	SL	NA
H/U	.117	.592	.592	.592	-	.500
D/U	.042	.592	-	.408	-	-

TABLE 32

Median Lead Concentrations (ug/g) for Odonates

STREAM	SITE	TRIP 1	2	3	4	5
	U	2.76	5.09	5.74	2.90	4.44
HO	H	1.87	2.65	4.63	2.13	6.21
	D	1.72	1.24	2.02	4.75	4.59
	U	2.74	1.31	2.33	2.26	1.62
BD	H	1.39	1.57	1.35	1.88	0.88
	D	2.87	1.52	2.33	2.31	3.01
	U	3.77	2.30	4.43	1.16	2.13
BR	H	1.57	1.94	4.37	1.03	3.03
	U	1.30	2.80	2.78	2.15	3.95
NO	H	1.41	11.28	1.83	1.05	2.37
	D	1.95	2.10	2.17	1.37	3.46
	U	0.55	0.84	1.02	1.44	1.80
SL	H	0.38	1.95	0.80	-	1.97
	D	0.58	-	1.03	1.33	1.09
	U	2.36	3.66	3.10	4.24	4.88
NA	H	1.90	2.88	4.63	4.53	4.73
	U	2.25	2.67	3.23	2.36	3.14
MEAN	H	1.42	3.71	2.94	2.13	3.20
	D	1.78	1.62	1.89	2.44	3.04

General temporal analyses showed a significant increasing trend in the H/U ratios over the course of the study. In other words, there was a significant trend towards increasing lead levels at highway sites in relation to upstream sites. Stream specific analyses indicated a significant increasing trend in the H/U ratio for stream BR.

Whole-body Fish

The analysis of whole-body metals concentrations was, like benthic invertebrate metals analysis, complicated by the fact that fish metals burdens are to some extent species specific (Martin 1983; Ney and Van Hassel 1983). This is especially true for zinc which is under at least partial homeostatic control (Bryan 1976). Different species from the same site often had significantly different metals concentrations. Unfortunately, no one species was present at every site on every trip. Although the statistical procedures employed were selected to be immune to this problem, the presentation of median metals values was not. Nonetheless, the mean of median fish whole body metals values across streams by site type are presented for reference, as to the range of concentrations encountered

TABLE 33

Odonate Lead Ratios

STREAM		TRIP 1	2	3	4	5
HO	H/U	0.678	0.521	0.807	0.735	1.399
	D/U	0.623	0.244	0.352	1.639	1.033
BD	H/U	0.508	1.198	0.579	0.834	0.541
	D/U	1.047	1.160	1.000	1.023	1.853
BR	H/U	0.416	0.843	0.985	0.884	1.422
NO	H/U	1.080	4.029	0.658	0.488	0.600
	D/U	1.494	0.752	0.781	0.637	0.877
SL	H/U	0.691	2.321	0.789	-	1.094
	D/U	1.055	-	1.015	0.924	0.606
NA	H/U	0.803	0.788	1.495	1.067	0.968
MEAN	H/U	0.696	1.617	0.886	0.802	1.004
	D/U	1.055	0.719	0.787	1.056	1.092

TABLE 34

Odonate Lead Significance Probabilities

I. General Spatial (Wilcoxon Rank Sum)

	TRIP				
	1	2	3	4	5
H ≤ U	.98	.08	.92	.94	.23
D ≤ U	.85	.77	.88	.85	.58

II. General Temporal (Page's Test)

H/U	D/U
.090	.282

III. Stream Specific Temporal (Theil-Sen)

	STREAM					
	HO	BD	BR	NO	SL	NA
H/U	.117	.500	.042	.883	-	.408
D/U	.242	.408	-	.592	-	-

(Table 35). As expected, there was quite a range in median zinc concentrations by species, less for cadmium and lead (Table 36).

As regards site type differences, the mean values for the pooled fishes show no major or consistent differences for zinc. Cadmium and lead levels appear to be higher at upstream sites than at highway or downstream sites. Mean cadmium and lead levels at highway sites appear to be increasing over the course of the study. However, the same is true for Cd and Pb at upstream sites, and for Cd at downstream sites.

Zinc.

General spatial statistical analysis revealed significantly higher fish whole body zinc concentrations at highway sites on Trips 4 and 5, and at downstream sites on Trips 2, 4, and 5 (Table 37), compared to upstream sites.

General temporal analysis showed a significant decreasing trend in the significance probabilities associated with the highway versus upstream general spatial tests for each trip. In other words, there was a significant decreasing trend in the probability that zinc levels in fish at highway sites was less than or equal to that at upstream sites. Stream

TABLE 35

Mean Fish Whole-body Metal Burdens (ug/g) by Site Type

		TRIP				
METAL	SITE	1	2	3	4	5
ZINC	U	91.7	119.0	115.0	111.4	113.9
	H	67.5	118.2	103.0	101.6	101.6
	D	82.2	139.7	123.9	126.4	107.9
CADMIUM	U	0.128	0.168	0.195	0.150	0.254
	H	0.071	0.108	0.148	0.145	0.177
	D	0.103	0.113	0.187	0.141	0.319
LEAD	U	1.08	2.17	1.29	1.34	1.86
	H	0.74	0.89	0.99	1.10	1.06
	D	0.94	0.85	0.68	0.64	1.06

TABLE 36

Median Fish Whole-Body Metals Burdens (ug/g) by Species

Species	n	Zn	Cd	Pb
Least brook lamprey	183	81.0	0.050	0.70
American eel	273	88.4	0.170	0.80
Pirate perch	91	91.6	0.190	1.17
Creek chubsucker	98	98.9	0.090	0.60
Tesselated darter	59	106.0	0.230	0.90
Bluegill	66	116.1	0.069	0.50
Bluehead chub	93	127.6	0.180	0.50
Creek chub	226	136.3	0.130	0.63
Blacknose dace	80	207.1	0.157	1.00
Eastern mudminnow	17	289.6	0.181	1.12

TABLE 37

Fish Whole-body Zinc Significance Probabilities

I. General Spatial (Wilcoxon Rank Sum; Fisher's)

	TRIP				
	1	2	3	4	5
H ≤ U	.72	.11	.52	.002	.001
D ≤ U	.77	.06	.19	.004	.009

II. General Temporal (Page's Test)

H/U	D/U
.042	.117

III. Stream Specific Temporal (Theil-Sen)

	STREAM					
	HO	BD	BR	NO	SL	NA
H/U	.117	.958	.500	.167	.042	.833
D/U	.167	.500	-	.500	.408	-

specific analyses indicated a significant decreasing trend in the p-values for the highway vs upstream pairing for stream SL.

Cadmium.

General spatial statistical analysis revealed significantly higher whole body cadmium concentrations at highway sites on Trip 4 and at downstream sites on Trips 2, 3, 4, and 5 (Table 38), compared to upstream sites. The general temporal analysis showed a significant decreasing trend in the significance probabilities associated with the downstream vs upstream comparison. There was no such trend for the H/U ratio. Stream specific analyses indicated significant decreasing trends in the H vs U p-values for stream SL and in the D vs U p-values for streams HO and SL.

Lead.

General spatial statistical analysis revealed that whole fish lead levels were not significantly higher at highway or downstream sites on any Trip (Table 39). In fact, lead levels were significantly higher at upstream sites than highway or downstream sites on Trips 2, 3, and 4.

General temporal analysis showed no trend towards decreasing p-values over the course of the study. Stream specific temporal analyses did show a significant trend towards decreasing p-values (i.e. increasing lead) at the

TABLE 38

Fish Whole-body Cadmium Significance Probabilities

I. General Spatial (Wilcoxon Rank Sum; Fisher's)

	TRIP				
	1	2	3	4	5
H ≤ U	.35	.80	.84	.001	.18
D ≤ U	.50	.075	.001	.001	.001

II. General Temporal (Page's Test)

H/U	D/U
.408	.080

III. Stream Specific Temporal (Theil-Sen)

	STREAM					
	HO	BD	BR	NO	SL	NA
H/U	.408	.592	.500	.833	.042	.438
D/U	.042	.167	-	.167	.008	-

TABLE 39

Fish Whole-body Lead Significance Probabilities

I. General Spatial (Wilcoxon Rank Sum; Fisher's)

	TRIP				
	1	2	3	4	5
H ≤ U	.63	.95	.99	.99	.85
D ≤ U	.80	.97	.97	.99	.46

II. General Temporal (Page's Test)

H/U	D/U
.675	.546

III. Stream Specific Temporal (Theil-Sen)

	STREAM					
	HO	BD	BR	NO	SL	NA
H/U	.500	.117	.625	.042	.180	.958
D/U	.500	.375	-	.500	.820	-

highway site for stream NO. Conversely, it also showed a trend towards increasing p-values (decreasing lead with respect to upstream) for stream NA.

Fish Tissues

Metals levels in fish tissues were subject to the same interspecies variations that plagued fish whole-bodies. Again, statistical tests were used that were immune to these potentially confounding effects. An additional problem encountered was a wide variation in metals levels in the livers of the various cyprinids. This was most likely due to the diffuse nature of the liver in these fish, and the extreme difficulty in extracting the entire organ, which is nonhomogeneous with respect to metals levels (Heit 1979).

American eels were the most numerous of the fish used for tissues analysis. Their organs were discrete and easily dissectable in toto. Their metals levels were less variable and are presented for reference (Table 40).

Liver Zinc.

General spatial statistical analysis revealed no significantly higher liver zinc concentrations at highway or downstream sites than at upstream sites (Table 41). In fact, liver zinc levels were significantly lower at highway sites than upstream sites on Trip 3. General temporal

TABLE 40

Median Eel Tissues Metals Levels (ug/g) by Site and Trip

TISSUE	SITE	TRIP 1	2	3	4	5
	U	--	145.4	167.2	134.7	150.1
Liver Zn	H	140.6	132.5	131.7	117.6	141.1
	D	142.1	114.6	122.7	141.4	132.4
	U	--	10.68	3.84	2.79	5.42
Liver Cd	H	2.04	4.49	3.05	2.29	4.23
	D	6.03	3.86	2.76	3.68	5.54
	U	-	2.61	2.90	4.13	4.61
Liver Pb	H	1.46	1.95	2.12	1.19	1.01
	D	3.60	3.20	1.07	1.12	0.87
	U	-	204.4	146.0	146.4	117.3
Kidney Zn	H	108.1	173.8	134.6	137.2	122.5
	D	133.7	157.7	146.5	157.8	100.2
	U	-	21.04	9.71	6.59	8.32
Kidney Cd	H	6.69	8.72	6.12	5.69	4.33
	D	3.50	10.32	9.05	7.24	3.00
	U	-	7.98	5.28	4.73	2.95
Kidney Pb	H	1.60	3.15	2.95	2.04	1.74
	D	2.62	9.31	2.00	1.07	0.81
	U	-	74.9	68.6	82.7	71.1
Bone Zn	H	61.6	73.3	77.5	71.1	81.6
	D	69.2	66.7	77.2	83.4	56.2
	U	-	7.31	7.74	8.21	9.86
Bone Pb	H	3.53	5.07	4.81	2.80	3.73
	D	10.33	11.47	2.67	3.28	4.58

analysis showed no trends towards increasingly higher liver zinc at highway or downstream sites over time relative to upstream sites.

Liver Cadmium.

General spatial statistical analysis revealed significantly higher liver cadmium levels at downstream sites than upstream sites on Trips 3 and 4 (Table 42). General temporal analysis showed no trends toward increasing cadmium levels at either highway or downstream sites, relative to upstream sites.

Liver Lead.

General spatial statistical analysis revealed no significantly higher liver lead levels at highway or downstream sites compared with upstream sites (Table 43). General temporal analysis showed no trend towards increasing liver lead levels at highway or downstream sites relative to upstream.

Kidney Zinc.

General spatial statistical analysis revealed that kidney zinc concentrations at highway and downstream sites were not significantly higher than those from fish at upstream sites (Table 44). General temporal analysis showed no trend towards increasing kidney zinc levels at highway or downstream sites relative to upstream.

TABLE 41

Liver Zinc Significance Probabilities

I. General Spatial (Wilcoxon Rank Sum; Fisher's)

	TRIP				
	1	2	3	4	5
H ≤ U	.63	.69	.96	.53	.82
D ≤ U	.80	.85	.75	.70	.91

II. General Temporal (Theil-Sen)

H/U	D/U
.592	.500

TABLE 42

Liver Cadmium Significance Probabilities

I. General Spatial (Wilcoxon Rank Sum; Fisher's)

	TRIP				
	1	2	3	4	5
H ≤ U	.13	.62	.57	.58	.42
D ≤ U	.11	.12	.06	.06	.14

II. General Temporal (Theil-Sen)

H/U	D/U
.500	.546

TABLE 43

Liver Lead Significance Probabilities

I. General Spatial (Wilcoxon Rank Sum; Fisher's)

	TRIP				
	1	2	3	4	5
H ≤ U	.85	.54	.41	.71	.91
D ≤ U	.20	.12	.19	.20	.60

II. General Temporal (Theil-Sen)

H/U	D/U
.592	.818

TABLE 44

Kidney Zinc Significance Probabilities

I. General Spatial (Wilcoxon Rank Sum; Fisher's)

	TRIP				
	1	2	3	4	5
H ≤ U	.55	.21	.64	.19	.78
D ≤ U	.61	.93	.42	.51	.76

II. General Temporal (Theil-Sen)

H/U	D/U
.592	.500

Kidney Cadmium.

General spatial statistical analysis revealed significantly higher fish kidney cadmium concentrations at downstream sites as compared to upstream sites on Trips 3 and 4 (Table 45). General temporal analysis showed no trend towards continually increasing kidney cadmium concentrations at highway or downstream sites relative to upstream sites.

Kidney Lead.

General spatial statistical analysis revealed significantly higher kidney lead levels at downstream sites than at upstream sites on Trips 2 and 4 (Table 46). General temporal analysis showed no trend towards progressively increasing kidney lead levels at highway or downstream sites as compared to upstream sites.

Bone Zinc.

General spatial statistical analysis revealed that bone zinc levels were not significantly higher at highway or downstream sites as compared to upstream sites on any Trip (Table 47). In fact, bone zinc levels were significantly higher at upstream sites than highway or downstream on Trip 2. General temporal analysis showed no trends towards increasing bone zinc levels at highway or downstream sites as compared to upstream.

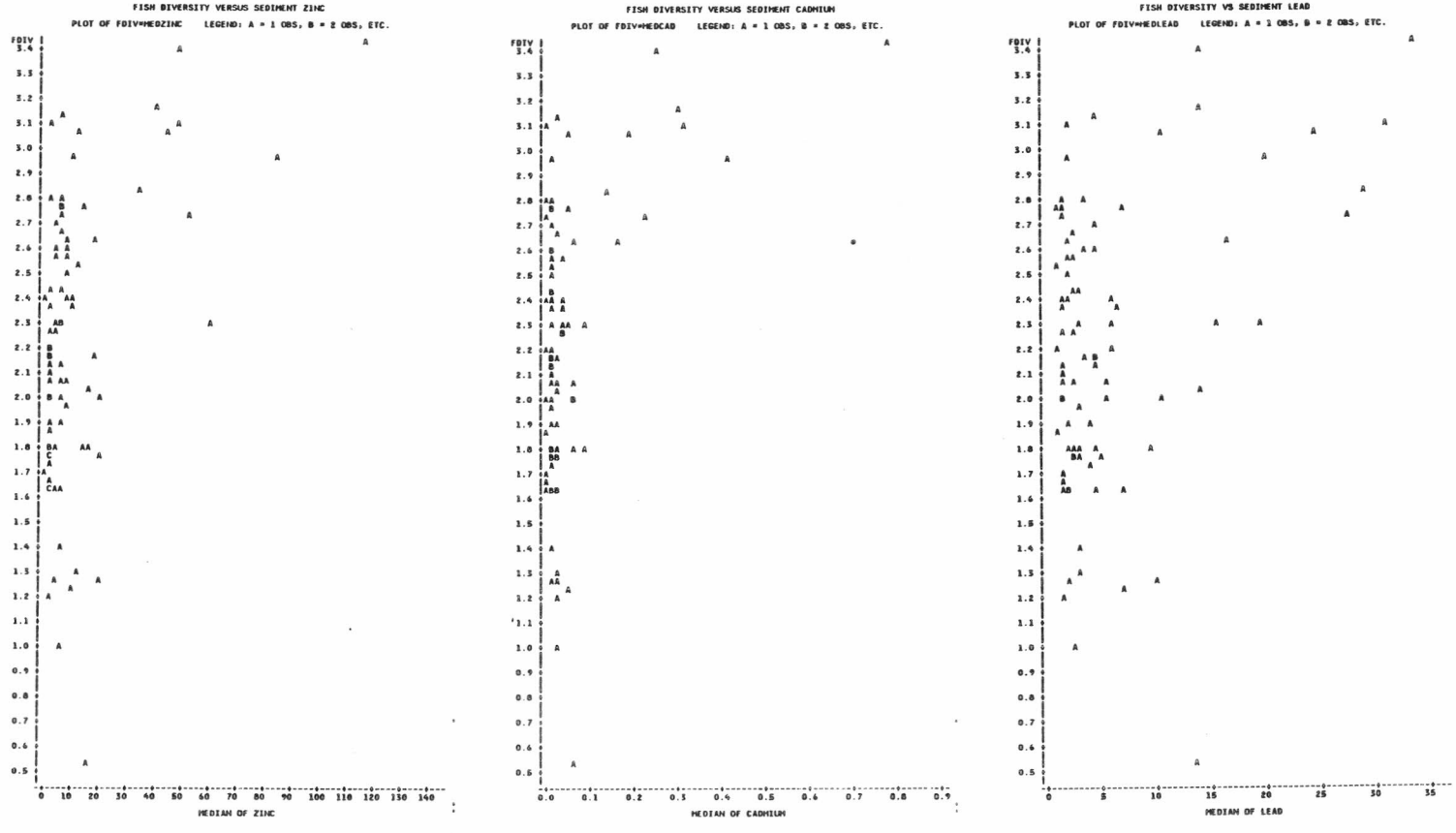


Figure 5. Fish Species Diversity Versus Median Sediment Metals Concentrations for the Study Sites.

diversity at sites of lower metals contamination (sites with sub-threshold levels) was greater than or equal to diversity at the more contaminated sites were 0.0013, <0.0001 and 0.0008 for Zn, Cd, and Pb respectively. The null hypotheses are resoundingly rejected.

Significant correlations were found between between fish diversity and the median sediment concentrations of all three metals (Table 69). The strongest correlation was between fish diversity and sediment zinc.

Fish Community Density

The scatter plots of fish density versus sediment metals concentrations (Figure 6) suggest that fish density was independent of sediment metals levels. There were no indications that fish density values were normally distributed around any one mean value. There were no significant correlations between fish density and the concentration of any sediment metal (Table 69).

Fish Community Biomass

The scatter plots of fish biomass versus sediment metals concentrations (Figure 7) suggest that fish biomass may, in some way, be related to sediment metals levels. The nature of the relationship, however, is not clear. Significant

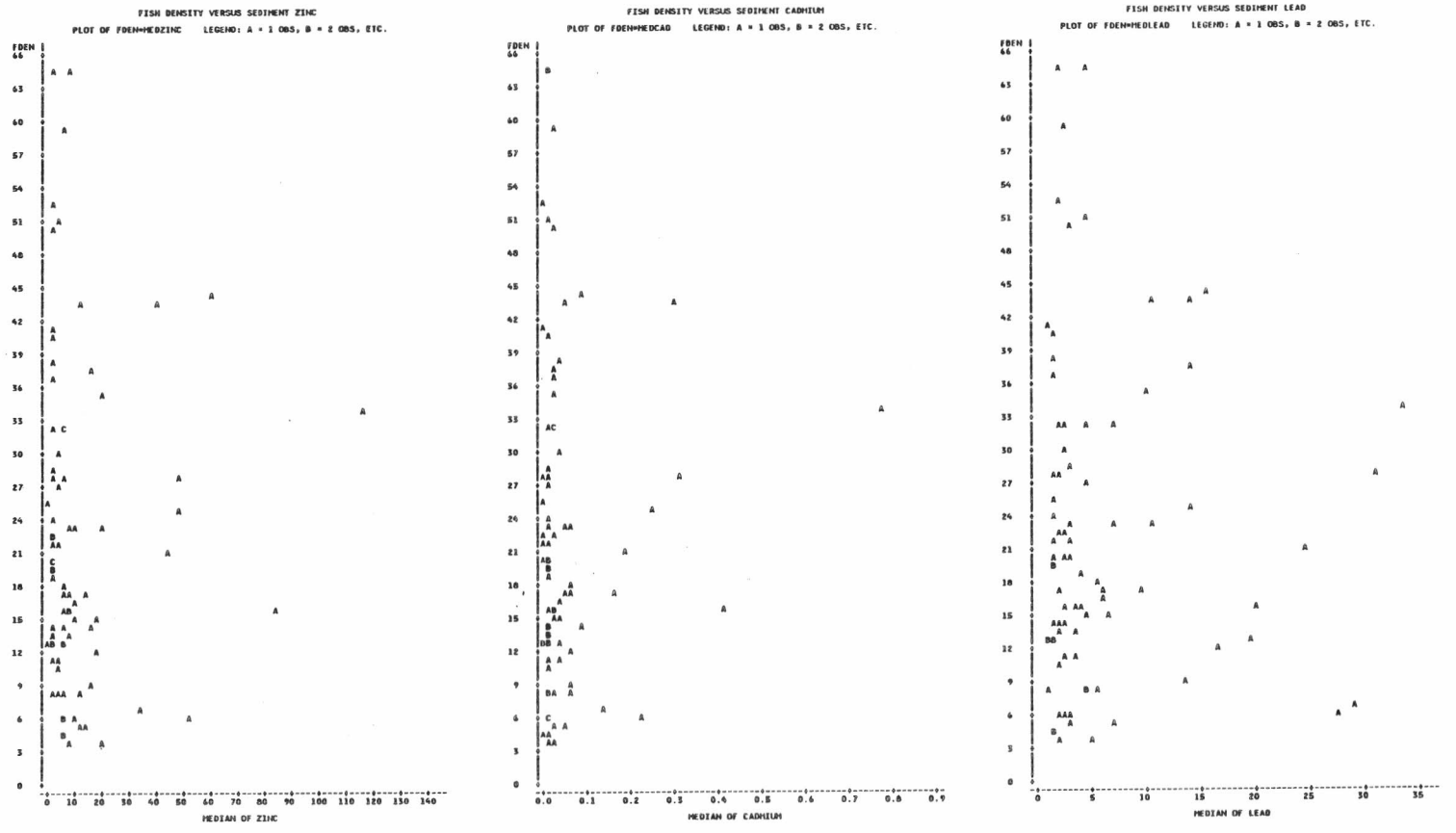


Figure 6. Fish Community Density (#/10m) Versus Median Sediment Metals Concentrations for the Study Sites.

positive correlations were found between fish biomass and sediment cadmium and lead levels (Table 69). The strongest correlation was between fish biomass and sediment lead.

I-95 Streams

Metals Contamination

Mean metals levels in most ecosystem components investigated were greater for I-95 streams than for I-295 streams (Table 70). The differences were least apparent in sediment and benthos metals levels, and most pronounced for fish whole bodies and tissues. As with the I-295 streams there was a large amount of interstream variability in metals levels. Mechump's and Walthall were the most contaminated streams, MM94.5 showed little contamination. One fact that is evident from inspection of the data is that neither of the two upstream sites is uncontaminated from the point of view of metals contamination.

Biotic Parameters

The biotic parameters for the I-95 streams have values that are indicative of more stressed environments than those of the I-295 streams. This is true both for benthos (Table 71) and for fish (Table 72).

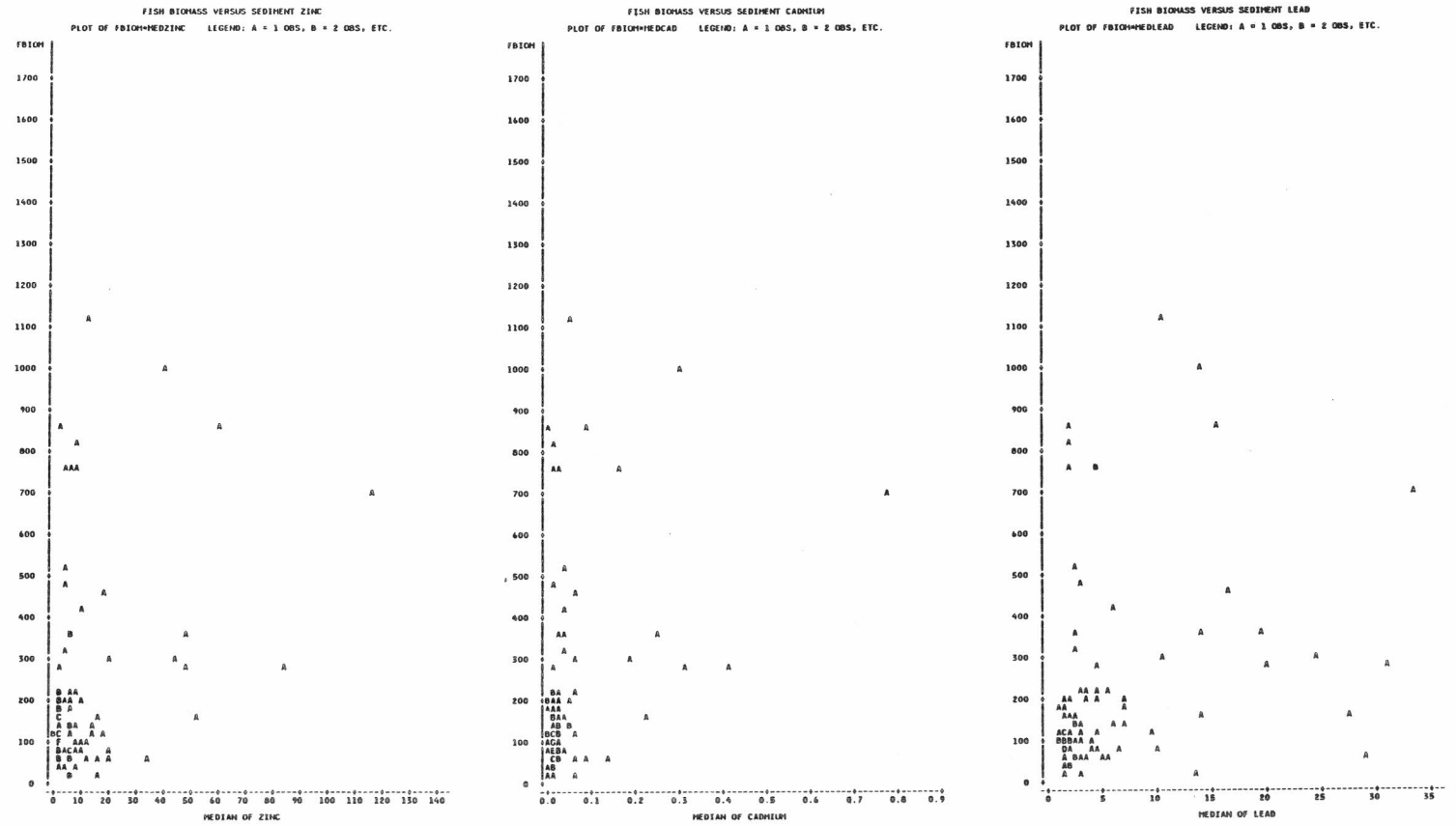


Figure 7. Fish Community Biomass (g/10m) Versus Median Sediment Metals Concentrations for the Study Sites.

TABLE 70

Median Metals Levels (ug/g) for I-95 Samples

SAMPLE	STREAM	SITE	METAL		
			Zinc	Cadmium	Lead
Sediment	Walthall	(U)	1.51	0.010	1.30
	Walthall	(H)	5.60	0.021	24.40
	Mechump's	(U)	4.64	0.022	12.14
	Mechump's	(H)	4.80	0.058	10.24
	Flippen	(H)	2.63	0.009	2.90
	MM 94.5	(H)	6.32	0.042	9.86
Odonates	Walthall	(U)	178.69	1.120	2.87
	Walthall	(H)	173.32	1.274	7.10
	Mechump's	(U)	107.67	1.324	34.26
	Mechump's	(H)	107.72	0.425	51.77
	Flippen	(H)	112.75	0.688	3.26
Whole fish	Walthall	(U)	162.14	0.732	5.70
	Walthall	(H)	134.61	0.925	4.66
	Mechump's	(U)	450.26	0.805	11.93
	Flippen	(H)	176.91	0.496	2.01
	MM 94.5	(H)	102.41	0.324	1.19
Liver	Walthall	(U)	189.70	14.560	1.34
	Walthall	(H)	95.61	9.760	4.70
	Flippen	(H)	113.08	9.250	2.10
	Kingsland	(H)	91.00	9.388	3.06
Kidney	Walthall	(U)	294.23	29.510	14.24
	Walthall	(H)	248.06	22.050	17.71
	Flippen	(H)	114.24	12.179	2.19
	Kingsland	(H)	207.97	24.880	8.13
Bone	Walthall	(U)	112.85	--	33.54
	Walthall	(H)	73.85	--	38.71
	Flippen	(H)	64.21	--	4.24
	Kingsland	(H)	79.61	--	20.05

TABLE 71

Benthic Invertebrate Biotic Parameters: I-95

Stream	Site	Diversity	Density[1]	AIPC[2]
Walthall	U	1.208	13	0.818
	H	1.974	68	0.900
Mechump's	U	1.491	12	--[3]
	H	1.041	46	1.000
Flippen	H	1.282	91	0.895
MM 94.5	H	3.055	104	0.152

[1] #/0.26m²
[2] The percentage of the aquatic insect community that was composed of chironomids.
[3] No insects present.

TABLE 72

Fish Community Diversity, Density, and Biomass: I-95

Stream	Site	Diversity	Density[1]	Biomass[2]
Walthall	U	1.531	10.00	165.46
	H	0.842	12.15	155.71
Mechump's	U	0.918	0.52	6.78
	H	0.722	0.86	28.71
Flippen	H	2.364	44.67	81.80
MM 94.5	H	1.192	4.00	21.83

[1] #/10m stream
[2] g/10m stream

Benthic Invertebrates.

Benthos diversity (Shannon Index) averaged 1.35 for sites upstream of I-95 and 1.838 for sites located at the highway. Values for individual sites are consistent with values for I-295 streams, with the exception of MM94.5, which had the highest diversity for any site in this study. It is unlikely that this high diversity was the result of metals contamination, since contamination of this site was rather average.

The percentage of the aquatic insect community that was composed of chironomids (AIPC) averaged 0.818 for upstream sites and 0.737 for highway sites for the I-95 streams. These compare to 0.625 and 0.671 for I-295 upstream and highway sites respectively. It should be remembered that both of the I-95 upstream sites were significantly metals-contaminated. The AIPC for the upstream site on Mechump's Creek could not even be computed, since no insects were present at all. The AIPC for the highway site on that stream was 1.00 (all insects present were chironomids). Only MM94.5 highway site had a AIPC of less than 0.90.

Benthic invertebrate density (#/0.26m^2) averaged 12.5 for upstream sites and 77.3 for highway sites. These values are

well below mean values for I-295 streams, which were 276.2 for upstream sites, and 166.2 for highway sites.

Fish Community Parameters.

Fish species diversity averaged 1.225 for I-95 upstream sites and 1.280 for highway sites. These values compare with 1.928 for I-295 upstream sites and 2.517 for I-295 highway sites. There was a wide range of diversity for the I-95 highway sites. Diversity ranged from 0.722 to 2.364, however three of the four highway sites had a lower species diversity than any recorded for the I-295 highway sites.

Fish community density for I-95 streams was also depressed, in relation to I-295 streams. Fish density (#/10m) averaged 5.26 for upstream sites and 15.42 for highway sites. These figures compare with 26.75 for upstream sites and 22.02 for highway sites of the I-295 streams.

Fish community biomass for I-95 streams was also lower than that for I-295 streams. Biomass (g/10m stream) averaged 86.12 for upstream sites and 72.01 for highway sites. This compares with 339.9 and 270.7 for upstream and highway sites of the I-295 streams. Severely reduced fish biomass was noted for Mechump's Creek, both upstream and highway sites. Of the other streams, two of the highway sites had biomass values within a normal range, judging from I-295 streams.

DISCUSSION

Water Quality

Water quality parameters for the six I-295 streams were quite similar overall. The streams were soft water, and mildly acidic to neutral. This makes them more susceptible to the toxicity of heavy metals than more hard-water, alkaline streams, because the metals are more apt to be in solution (Hem and Durum 1973; Freedman et al. 1980; Houba and Remacle 1982). The only notable difference between site types was that conductivity was often higher at highway sites, indicating another possible constituent in the water at those sites. However, there was no trend for turbidity to be greater at those highway sites sites with higher conductivities.

Interpretation of Metals Contamination

Water

The concentrations of zinc, cadmium, and lead in natural waters are low. Concentrations for unpolluted flowing fresh waters average from 0.01 to 0.1 mg/l for zinc (Taylor et al. 1982; Martin et al. 1980b), from 0.01 to 0.1 ug/l for

cadmium (Martin et al. 1980a), and from 5-7 ug/l for lead (Demayo et al. 1982). The metals levels found in this study are within these ranges, or lower.

There are three methods by which metals were expected to enter the water of roadside streams: runoff, aerial deposition, and sediment leaching. These three methods are associated with two characteristic patterns of accumulation. The first is the highly variable contamination associated with runoff events. The second is the lower-level, less variable rate of contamination associated with a relatively constant aerial deposition, and/or release from the sediments.

The results in this study indicated mainly negligible differences in water metals concentrations among the site types. When the data are examined with respect to the expected patterns of contamination described above, there is nothing present to confirm the existence of contamination of either source. In review, the only significant site type differences that were consistent with the hypotheses was that zinc was higher downstream than upstream on Trip 3, and cadmium was higher at the highway than upstream on Trip 5. This sporadic nature of significant results is inconsistent with the method of contamination and is, as a result of dubious consequence.

The lack of documentation of the runoff scenario for metals introduction is not unexpected. To properly assess this type of contamination, it would have been necessary to have a sampling regime oriented around collecting samples during periods of highway runoff. Because of the unpredictability of these occurrences, the distance to the study area, and the incompatibility of these periods with the sampling for other elements of this study, this was not attempted.

The significant increase in the H/U lead ratios over the course of the study is consistent with a steady release of lead from the sediments, if the amount of lead in the sediments increases through time as well. In addition, it is not inconsistent with the aerial deposition mode of contamination, given the pattern of vehicular traffic increase. It should probably be noted that these two methods are not mutually exclusive and probably occur together. Inspection of the individual ratios makes it clear that this increase (whatever its source), though statistically significant, is of very small magnitude, and probably insignificant from a biological standpoint.

The magnitude of the water metals concentrations found in the study streams in relation to EPA ambient water quality criteria warrants discussion. Zinc concentrations, with

three exceptions, did not exceed the criteria. However, all but twelve (out of 71) of the water cadmium concentrations, and all but one of the lead concentrations exceeded EPA Ambient Water Quality Criteria for chronic exposure. It appears to me that, at least for water of these hardnesses, that the present criteria may be unrealistically stringent. I base this on several observations. First, criteria were exceeded at all site types, upstream, highway, and downstream. In addition, the metals levels found in these streams were low to average, in comparison to nation-wide average values. Further evidence for the excessively stringent nature of the criteria comes from a study of USGS benchmark streams. These streams have been selected for their pristine nature and representativeness of unspoiled conditions. However, 76% of them were above the EPA criteria for chronic exposure for copper (Buikema and Cherry 1982). As a result, I attach no particular significance to the violations of EPA criteria in the study streams.

Sediments

Naturally occurring metals levels in sediments vary considerably with the underlying geology of the region. A review of natural sediment zinc concentrations by Taylor et al. (1982) show a range of zinc concentrations of from 2.5

to 670 ug/g. Forstner (1980) reported cadmium concentrations in the sediments of unpolluted rivers to range from 0.14 to 0.25 ug/g. Levels in polluted sediments ranged up to 50,000 ug/g. Natural lead levels in river sediments range from 8 to 274 ug/g. They can reach as high as 6000 ug/g in polluted sediments (Demayo et al. 1982). Feltz (1980) reports the results of sediment metals analyses for Potomac River tributaries. These levels (particularly the lower levels, since the upper ones may indicate pollution) should be more representative of those expected for Richmond area streams, given the geologic similarities. He reports ranges of 4.7 to 220 ug/g Zn, <0.0 to 0.81 ug/g Cd, and <0.0 to 200 ug/g Pb. Sediment metals concentrations found in this study were within these ranges.

Significant differences were found in sediment metals levels that were in accordance with the hypothesis that metals levels would increase at highway and to a lesser degree, downstream sites, in relation to upstream sites. Zinc levels were significantly higher at highway sites on three of the five Trips, cadmium on four of five, and lead on three of five Trips. The only significant difference found among downstream and upstream sites was that zinc levels were higher at downstream sites on Trip 1. However, inspection of the individual and mean values shows that in

most cases the downstream metals levels were intermediate between upstream and highway levels. It should also be noted that, since the sample size for the downstream sites was less than for upstream and highway sites (n=4 versus n=6), a larger difference in metals levels would have been required for a statistically significant difference between downstream and upstream sites to be achieved.

The pattern of accumulation of these metals in the sediments followed a somewhat different course than was expected. Rather than a gradual progressive increase, metals levels were higher at highway sites than some upstream sites from the start. Mean concentrations at highway sites did increase, with zinc, cadmium, and lead levels peaking after about one year (Trip 3), at 635%, 1106%, and 474% respectively, of upstream reference values, and then remained about the same or even declined. This suggests that the temporal pattern of metals contamination of roadside stream sediments follows a sort of dynamic plateau, with the time required to progress from background levels to plateau levels short, on the order of months.

This assertion is supported by several aspects of this study. The first sampling trip reported in this study took place in March, 1981. Unfortunately for this study, the majority of I-295 opened ahead of schedule, in the fall of

1980. This allowed sufficient time for metals concentrations to rise above background levels as a result of automotive traffic even before the first sampling trip. On a preliminary sampling trip made in November 1980 to streams HO and BD, metals levels were much lower than those found at highway sites on the first reported trip to those sites (July, 1981), and in line with those found at upstream and downstream sites. Zinc concentrations at highway sites on this preliminary trip amounted to only 17% and 42% of those found on the first reported trip for streams HO and BD respectively. Cadmium levels on this preliminary trip were not lower, being 106% and 255% of those found on the subsequent trip. Lead levels, like zinc, were much lower on this early trip, both being 12% of what was found on the first reported trip. Thus, at least for zinc and lead, a more dramatic rising part of the contamination curve is not seen because of the length of time between highway opening and the initiation of the study.

Other evidence for the dynamic plateau hypothesis of roadside stream sediment contamination comes from the I-95 streams. Because of the higher traffic densities (about four to five times) on I-95, it was expected that these sediments would be much more contaminated than those from the I-295 streams. Van Hassel et al. (1980) found significant positive

correlations between roadside stream sediment metals levels and traffic densities. However, sediment metals levels at these sites were not particularly high with respect to the I-295 streams. Zinc levels found at I-95 highway sites would have been average to low for the I-295 streams. Sediment cadmium levels were equivalent to levels found at I-295 highway sites. Only lead levels were equal to some of the highest levels found for I-295 highway sites. These levels are inconsistent with the highway traffic density correlations, but not with the dynamic plateau hypothesis, in which spot-checks of metals contamination are expected to show varying metals levels.

The dynamic plateau would be achieved in the following manner. Metals accumulate in stream sediments at a rate that is proportional to traffic density. However, the same runoff that can deposit metals can also, at higher velocities, remove these metals from the roadside stream environment (Forstner 1980; Benes et al. 1985). It is also conceivable that these metals can be removed from stream sediments by a steady leaching, the natural consequence of their input into the water column by this method, as mentioned earlier, or more rapidly in response to water quality changes such as pH excursions (Paul and Pillai 1983a).

Rainfall records from the Richmond Airport were examined in an attempt to gain further understanding of the temporal pattern of sediment metals accumulation noted at the I-295 sites. The highest sediment levels (Trip 3) were coincident with a rather lengthy drought period, during which scouring of the sediments may not have occurred. Following this Trip, rainfall amounts returned to about normal levels, and sediment metals decreased somewhat. With respect to the I-95 sediment levels, examination of these records revealed that over 10 cm of rainfall occurred in the Richmond area in the two weeks preceding the I-95 sampling trip. Had this rainfall not occurred, it is likely that the sediment metals concentrations found there would have been higher.

The result of a relatively constant deposition overlaid on a variably occurring scouring, is that metals levels would not continually increase, but rather rise and fall as a result of environmental factors about a dynamic equilibrium. The equilibrium point would be expected to be positively correlated with traffic density (the levels build faster, and thus rise higher before they are reduced). Thus one would expect that, on average, metals levels would be higher on streams influenced by more automotive traffic, but on any given day they need not be so.

Another interesting result of the study was the large amount of interstream variation in the extent to which metals accumulated in stream sediments. Two of the six I-295 highway sites showed large increases in the metals levels over upstream sites, two moderate, and two minor, if any increase. The streams that showed the largest increases had characteristics that would intuitively seem to promote contamination. They were the closest to the traffic lanes, they had the slowest water velocities (which would allow suspended metals-bearing particulate matter to settle out), and they had less vegetation than the other streams between the road surface and the stream to intercept airborne metals-laden dust. The average traffic density did not seem to be much of a factor; one of the two most contaminated streams had about 50% less traffic crossing it than the less contaminated streams, and the second highly contaminated stream had traffic densities equal to the other streams. The streams that did not show major increases in sediment metals were farther from the road surface, generally had higher water velocities, and often had more vegetation between the road and the stream surface. Because of the apparent relation to local topographic features, it should be possible to predict a priori, with some confidence, the relative magnitude of the increases to be expected.

Benthic Invertebrates

Reports of the concentrations of heavy metals in benthic invertebrates are not as numerous as those for fish. Enk and Mathis (1977) reported the cadmium and lead levels of four taxa of benthic invertebrates from an unpolluted Illinois stream. Mean cadmium values (wet weight) ranged from 0.53 ug/g for Hydropsyche to 1.54 ug/g for the damselfly Agrion. Mean lead concentrations (also wet weight) ranged from 6.88 ug/g for the mayfly Isonychia to 12.59 ug/g for Agrion. It is difficult to relate these concentrations to the levels found in this study, because the relationship between wet weight and dry weight for benthic invertebrates is not known to me, and probably varies widely among different taxa.

Anderson (1977) reports the metals concentrations (dry weight) of three genera of odonates from a mixture of unpolluted and polluted sites. The ranges of the mean values were 75.2 to 183.4 ug/g Zn, undetectable to 0.50 ug/g Cd, and ≤4.0 to 23.7 ug/g Pb. These values are, for the most part, equivalent to levels found in this study, although mean cadmium values at highway sites often exceeded 0.50 ug/g.

Metals concentrations in odonates exhibited changes that were consistent with contamination by the highway. This was true for both site type differences and for temporal

changes. However, visual interpretation of any trends in the individual and mean metals levels is impeded somewhat by species differences in metals accumulation. This is especially true for zinc. Odonate cadmium levels at highway sites seemed to follow the pattern seen in sediment contamination, peaking around Trip 3. Benthos generally had higher zinc and cadmium levels than did sediments. Lead levels were generally lower.

In review, odonate zinc levels were significantly elevated over upstream reference levels at highway sites on Trip 4, and downstream sites on Trips 1, 3, and 4. Cadmium levels were higher at highway sites on Trips 2 and 3, and downstream sites on Trips 2, 4, and 5. Lead levels were significantly higher at highway sites on Trip 2. In contrast, lead levels were significantly higher at upstream sites on Trip 1.

The fact that downstream sites were higher in relation to upstream sites than the highway sites, and relatedly the failure to find more significant differences between upstream and highway sites is probably due to the fact that downstream versus upstream comparisons were limited to the four streams that had downstream sites. The reason that this would make a difference is that the two upstream sites that were not used in the comparisons (BR, NA) were contaminated

by metals above background levels, by as yet unknown sources. For example, median Cd and Pb concentrations of aeshnids (the most abundant family analyzed) at upstream sites on BR and NA averaged 0.93 and 2.50 ug/g respectively, as opposed to 0.24 and 1.96 ug/g for the remaining four upstream sites. This contamination was not known before the study, and only became apparent well into it. The result is that highway site contamination is assessed against against higher upstream reference metals levels than are downstream sites. An extension of this fact is that highway versus upstream tests are more conservative than they should be, and that more differences would probably have been found had these two upstream sites not been contaminated. This phenomonon will be encountered again with fish whole bodies and tissues.

This unexpected contamination does not affect the temporal tests, either general or stream specific, because they are based on changes in the ratios between highway and upstream sites. Indeed, the general temporal test for odonate lead found a significant increase in the lead levels at highway sites through time.

Odonate zinc levels found at the I-295 highway sites were similar to those found for the I-95 highway sites. Overall mean values for the former were 127.2 ug/g as opposed to

131.3 ug/g for the latter. Again, due to homeostatic control and, to a lesser degree, species differences, this lack of differences is not unexpected. Cadmium levels in odonates at I-95 highway sites were within the range (albeit the high end) of those found at the I-295 streams. The mean of the median values for I-95 odonates was greater than 25 of the 30 median highway site values for I-295. However, the mean value for I-95 odonates was seven times the mean value for odonates at the I-295 highway sites over the course of the study. Apparently, the greater contamination at I-95 highway sites was reflected in higher cadmium and lead levels in macrobenthos there.

Whole-body Fish

Because of species-specific differences in metals concentrations, and the ability of fishes to accumulate these metals, especially cadmium and lead, in waters contaminated by metals (Atchison et al. 1977; Chernoff and Dooley 1979), a wide range of whole-body metals concentrations have been reported in the literature. The U.S. Fish and Wildlife Service (1984) reviewed published reports of whole-body metals contamination and report ranges (for various, unnamed species) of 17-472 ug/g Zn, undetectable to 21.36 ug/g Cd, and undetectable to 49.7 ug/g

Pb. May and Mckinney (1981) report the results of metals (but not zinc) sampling in fishes from 117 sampling stations nationwide. They propose some critical values for metals in fish above which the water that the fish came from would be suspected of being polluted. These were computed as the 85th percentile of the distribution of the concentrations gathered nationwide. After converting their wet weight concentrations to dry weight concentrations (ug/g wet weight = 4 x ug/g dry weight), their criteria are 7.0 ug/g and 28.1 ug/g for cadmium and lead, respectively. The concentrations of metals in fish whole-bodies in the present study were well below these levels, averaging less than 0.2 ug/g Cd, and less than 1.2 ug/g lead.

Fish whole-body metals concentrations increased markedly through time at I-295 highway sites. This was apparent for cadmium and lead even from the mean values of all fish pooled regardless of species. Zinc means did not show an increase through time at highway sites, perhaps because of its homeostatic control and/or interspecific differences in its accumulation. The fact that mean whole body concentrations of Cd and Pb increased in fish from upstream sites as well as highway sites indicates that either the zone of contamination around the highway was larger than reported (Laxen and Harrison 1977), or that some degree of

fish movement may have been occurring between the highway and upstream sites on at least one of the streams. If the former were happening, it would be expected to be reflected in increased metals concentrations in sediment and benthos at the upstream sites. Such a trend was not found. It could be that, under some streamflow conditions, these small fish were able to transcend the seeming barriers between highway and upstream sites.

The statistical tests that were used took the species differences into account and should have been, as a result, quite powerful in detecting site type and temporal differences. In review, whole-body zinc levels significantly greater than upstream reference levels were found at highway sites on Trips 4 and 5, and downstream sites on Trips 2, 4, and 5. Cadmium levels were significantly higher at highway sites on Trip 4, and at downstream sites on Trips 2, 3, 4, and 5. Lead levels were not significantly higher than upstream levels at highway and downstream sites on any Trip, and in fact, were actually lower on three Trips.

General temporal trends indicating increasing whole-body metals concentrations were found for highway sites for zinc, and for downstream sites for cadmium. No general trends were found for lead. Stream specific trends indicating increasing

metals were found for zinc and cadmium at the stream SL highway site, cadmium at downstream sites on streams HO and SL, and lead at highway sites on stream NO.

The lack of significantly higher lead levels at highway sites, and even more, the significantly lower levels found there was surprising. This finding may be due to the abnormally high Cd and Pb levels found in fish whole bodies at two of the upstream sites (BR, NA). Whole-body eel Cd and Pb concentrations at those sites averaged 0.48 and 2.39 ug/g, as opposed to 0.15 and 1.26 ug/g for the other upstream sites. This relates back to the unexpected contamination of these sites discussed earlier. Even if it had been possible to use the ratio approach on the fish whole bodies (it was not, because of species differences) significant temporal trends may not have been found since increases occurred at upstream sites as well as highway and downstream sites. The existence of increasing trends in whole body lead (and cadmium as well) levels is strongly suggested by the pooled whole body metals means by Trip.

Whole body metals concentrations (especially cadmium and lead) of the I-95 fish were much higher than those found for the fish found living alongside I-295. Mean values for I-95 whole-body zinc, cadmium, and lead were 1.4, 4.5, and 2.7 times as high as those found in fish the I-295 fish.

Apparently, higher traffic densities over longer periods of time cause fish whole body metals levels to be increased. This is in accordance with earlier findings of higher whole-body metals concentrations in metals contaminated environments (e.g Atchison et al. 1977; Chernoff and Dooley 1979; Van Hassel et al. 1980).

Fish Tissues

Literature reports of tissues metals concentrations indicate that species differences exist (MacFarlane and Franzin 1980; Martin 1983), and that concentrations in the same species can vary widely, depending on where the fish were taken. Cowx (1982) reported a mean liver zinc concentration of 255 ug/g in Salmo trutta from an unpolluted North Wales lake. Roline and Boehmke (1981) found mean liver zinc concentrations in the same species in a metals polluted area to average 700 ug/g. Martin (1983) found liver zinc concentrations in Lepomis macrochirus taken from a zinc polluted river to be about 100 ug/g. Examination of these and other reports (Pagenkopf and Newman 1974; Salanki et al. 1982; Paul and Pillai 1983b; Saltes and Bailey 1984) suggest the following ranges of tissues metals concentrations in fish from uncontaminated waters: liver Zn, 50-300 ug/g; liver Cd, undetectable to 1.5 ug/g; liver Pb, undetectable

to 3.5 ug/g; kidney Zn, 100-500 ug/g; kidney Cd, undetectable to 3.0 ug/g; kidney Pb, undetectable to 6.0 ug/g; bone Zn, 75 to 300 ug/g; and bone Pb, undetectable to 4.0 ug/g. These concentrations were frequently exceeded in this study, indicating contamination above background levels was occurring.

In this study, significantly higher metals concentrations at highway sites relative to upstream sites were found in only one instance (out of 40 comparisons): bone lead was significantly higher (although barely, $p=0.10$) at highway sites on Trip 5. Conversely, significantly higher tissues metals levels were found at upstream sites on three occasions. Downstream versus upstream comparisons of tissues metals concentrations fared better with respect to expectations. Tissues metals levels were higher downstream than upstream on six occasions. On many other instances they approached significance ($0.15<p<0.10$ on seven occasions). In only one instance were upstream tissues metals levels significantly higher (and then only barely so) than downstream tissues metals levels.

The failure of the highway sites to show contamination in relation to the upstream sites can again probably be traced to the unexpected contamination found at two of the upstream sites. Cadmium and lead levels in the tissues of eels from

streams BR and NA averaged two to five times higher than those from eels collected from the other upstream sites. This problem was of greater than normal magnitude from the standpoint of tissues metals analysis because these sites (especially NA) produced a larger number of fish for tissues analysis than other upstream sites. In addition, because tissues selectively concentrate these metals, the contamination was more pronounced over background levels vis a vis the other sample types (e.g. sediment).

Mean tissue cadmium and lead concentrations in tissues of the I-295 highway site eels were low in relation to those found for I-95 highway site eels. Mean values at I-95 were from about two to five times higher than those found in the corresponding tissues for I-295 eels. The greatest difference was found for bone lead. Differences in zinc levels in tissues between the two highways was mixed. Liver zinc was higher at I-295, kidney zinc at I-295. Bone zinc was essentially the same at both highways. These findings are consistent with the findings of other researchers (Weatherly et al. 1980; Saltes and Bailey 1984) that the factors that regulate zinc accumulation in fishes are poorly understood, and its interpretation is perplexing, at best.

The higher levels of cadmium and lead found in the tissues of some of the I-295 fish are indicative of sites

that are contaminated by metals. The concentrations of these metals in the streams along the more heavily travelled I-95 were even higher. Although the significance of these high metals burdens from an ecological standpoint is unclear (Ney and Van Hassel 1983), their presence should indicate the possibility of environmental harm.

Interpretation of Biotic Parameters

Benthic Invertebrate Diversity

Benthic invertebrate diversity has been widely used as a tool in water quality investigations. Cairns (1977) called the diversity index "probably the best single means of assessing biological integrity in freshwater streams and rivers." "Criteria" have even been proposed (Wilhm and Dorris 1968) whereby streams with diversities of less than one (Shannon Index) are, by definition, heavily polluted. If the diversity is between one and three, the stream is considered moderately polluted, and if the diversity is over five, the stream is clean. Wilhm and Dorris (1968) also reported lowered diversities as a result of organic wastes, oil field brines, oil refinery effluents, total dissolved solids (as measured by conductivity), and storm sewer outfalls. Roline and Boehmke (1981) found depressed macroinvertebrate diversities at sites located below heavy metal inputs to the Upper Arkansas River, Colorado.

Recently however, some researchers have questioned the usefulness of benthic macroinvertebrate diversity for the detection of chemical pollution (Godfrey 1978; Moore 1979; Chadwick and Canton 1984). Sloof (1983) reasoning from toxicological considerations, stated that "the reliablity of using biological systems based on macrobenthos distribution to classify surface waters polluted with a variety of chemical pollutants should be seriously doubted." Although conceding the usefulness of biotic parameters in organic enrichment studies, and instances where only one chemical pollutant is involved, he concluded that the differential toxicity of each of the chemical pollutant results in there being no tolerant or intolerant organisms, and hence no selective toxicity in the face of multiple chemical toxicants. However, he looked at a wide range of chemical pollutants (e.g. heavy metals, phenols, trichloroethylene to name a few). It seems likely to me that the differential toxicity should be much less within more closely related groups of pollutants (within the heavy metals, or within the phenols).

In this study, benthic invertebrate diversity did not respond to the differential metals contamination of the sites. Benthic invertebrate diversity was not significantly lower at highway sites than at upstream sites on any Trip.

In addition, there were no differences between downstream and upstream diversity. There was a significant trend towards decreasing diversity at downstream sites relative to upstream sites, but this trend was not found for highway sites, where, as a result of their greater contamination, it would have been expected to show first.

Further indication of the inadequacy of benthic invertebrate diversity to detect metals contamination in this study is provided by the diversity found on the I-95 streams. Median diversity at the I-95 highway sites averaged 1.838, as opposed to 1.410 over the course of the study at the I-295 highway sites. If one does not use the extraordinarily high value at one I-95 stream (3.06 at MM94.5), the mean benthic invertebrate diversity at I-95 streams becomes 1.432, virtually identical to the value for the I-295 highway sites.

However, the most conclusive evidence for the inadequacy of the benthic invertebrate diversity as an indiactor of metals contamination in this study comes from the plots of the diversity versus sediment metals levels, and the lack of any correlation between the two variables. In this study, benthic invertebrate diversity appeared to be normally distributed around a single mean, and independent of sediment metals levels.

I think the reason why benthic invertebrate diversity did not respond as expected (or at all, for that matter) in this was that of insufficient taxonomic differentiation. I used a mixed diversity index, as opposed to a species diversity index for benthic invertebrates. Insects were identified to genus, with the exception of chironomids which, due to the difficulty of identification, were only identified as such (i.e. to family). Non-insects were identified to class. Although this practice is not uncommon, given the taxonomic nightmares posed by the chironomids, I think the information lost by doing so was precisely the information needed to identify the effects of this low level contamination.

Hughes (1978) investigated the effect of different levels of taxonomic differentiation on diversity indices calculated for ten sites with differing levels of pollution. Consistent trends were evident in which diversity was always higher and showed greater changes with respect to the differential pollution of the sites with increasing taxonomic differentiation.

Aquatic Insect Percent Chironomids

Winner et al. (1980) in studying two second-order Ohio streams found a predictable graded response of macroinvertebrate community structure to metals pollution.

As copper contamination increased, so did the proportion of the aquatic insect community that was composed of chironomids (midge larvae). This change occured presumably because chironomids are more resistant to metals pollution than other aquatic insects. This apparently metals-specific index was quickly embraced within the framework of this study. As it turned out, the results warranted its inclusion.

In the hypothesis testing part of the I-295 study, the AIPC showed significant differences between site types on only one occasion. This is not unexpected in retrospect, given the contamination of two of the upstream sites, and the great variability in which the highway sites became metals-contaminated. The general temporal tests might have been expected to fare better, since the ratios were immune to anything but changes from originally occurring conditions. In addition, Page's test incorporated blocking by stream, which negated the obfuscating effects of the interstream variation. These tests seemed to perform better, the p-value for the H/U general test was 0.110, approaching significance. However, it should be remembered that the sediment metals themselves did not continually increase, but rather plateaued. Given that, there is no reason to expect the AIPC to continually increase.

An additional reason (that would operate concurrently with the previous) may be that because the AIPC was naturally high in these streams to begin with, it had little room to increase. Winner et al.'s (1980) uncontaminated sites had AIPC's of less than 0.10. At their contaminated sites it increased to 0.75 to 0.80. In this study, on the one stream (BR) where AIPC was relatively low (<0.50), a significant increasing trend was found at the highway site relative to the upstream site.

The AIPC at the I-95 highway sites provides further insight into the interplay between metals contamination and the AIPC, and at the same time, documentation of the effects of a greater traffic density on a biotic parameter of a roadside stream. AIPC at the I-95 streams ranged from 0.152 to 1.00. The lower number occurred at the atypical MM94.5 stream (which also had the highest benthic invertebrate diversity). This stream is a small stream, located relatively far from the traffic lanes and is situated such that it receives little highway runoff. It was not particularly highly contaminated even with respect to the I-295 streams. If this one stream is omitted from consideration, the mean AIPC becomes 0.932, with the lowest value being 0.895.

Examination of the relationships between sediments and AIPC showed AIPC to be significantly correlated with sediment cadmium and lead levels. However the Spearman correlation coefficients were low, both being around 0.275. As noted, this is probably because the relationship is nonlinear. The scatter plots suggest a threshold-like response, and intuitively, that is what I would expect. It seems only reasonable that below a certain threshold level, that metals levels are not likely to exert an influence on AIPC.

Benthic Invertebrate Density

Benthos density has been suggested as a good index of metals pollution, especially by those who reject benthic invertebrate diversity indices for the same purpose (e.g. Moore 1979; Kraft and Sypniewski 1981; Chadwick and Canton 1984). The general finding of these researchers is that as sediment metals increase, the density of benthic invertebrates decreases. In a study with a direct bearing on this one, Smith and Kaster (1983) found no significant differences in benthic invertebrate densities in a Wisconsin stream, that could be attributed to rural highway runoff. However, traffic densities were low (7000-8000 vehicles per day), and the streams were hard-water (alkalinity 350 mg/l as CaCO3).

With respect to the I-295 hypothesis testing, benthos density did not differ consistently among site types, although it did average 66% higher at upstream sites compared to highway sites. The only significant departures from upstream reference levels were for downstream sites on Trips 1 and 3. No differences were found at highway sites where they were more expected, due to the higher metals levels. More disconcerting however, was the significant increasing trend in benthos density at highway sites relative to upstream sites, in direct opposition to what was expected.

The benthic invertebrate densities on the I-95 streams in relation to the I-295 streams bore out expectations. Average benthic invertebrate density for I-95 highway sites was 47% of that at I-295 highway sites. I-95 upstream sites, which also had significant metals contamination had an average median invertebrate density of only 8% of those found at I-295 highway sites.

Examination of the relationships between sediment metals levels and benthos density indicate that there was some kind of relationship between benthos density and sediment metals levels. However the nature of the relationship is not clear. The scatter plots did not indicate a threshold like response. Spearman correlation coefficients indicated

significant positive correlations between sediment metals levels and benthic invertebrate densities. As mentioned previously, this is directly opposed to the findings of the other studies.

A possible explanation for the increasing trend found for benthos densities at the highway sites, and the positive correlations between benthos densities and sediment metals is that differential toxicity to invertebrate predators allowed the populations of invertebrate prey to increase. The low frequency of occurrence of predators on chironomids (principally odonates) in the samples made it impossible to determine whether indeed this was occurring. Another explanation, that could operate concurrently, is that metals exposure resulted in an enhancement of the detrital pathway (Hendrix et al. 1982), thus increasing the food base, and allowing chironomid numbers to increase. Selby et al. (1985) found that populations of pool dwelling chironomids increased as a result of chronic subacute cadmium exposure. Because of the large percentage of chironomids in the streams of my study, an increase in their numbers may have a greater effect on total benthic invertebrate densities than any decrease in intolerant organisms.

Another possible explanation for the failure of benthos densities to respond as expected (i.e. decrease), was that

the variability among samples taken at the same site (the replicates), and among sites, was too great in relation to the sample sizes (n=3). Raleigh and Short (1981) based on actual field sampling with the same type of sampler in a Colorado stream estimated that eight samples would be required at one site to get population estimates within a 10% standard error of the mean. Such intensive sampling was beyond of the scope of this study. However, the fact that differences (i.e. increases) were found tends to discredit this line of reasoning, since high variability would preclude the finding of differences in both directions.

I think the main reason that benthos densities did not decrease is that sediment metals may not have increased to a level sufficient to reduce them. The metals levels in the afore-mentioned studies in which benthic invertebrate density decreases were found were much higher than those experienced in this study. For example, Moore (1979) worked with sediments having lead and zinc levels up to 850 and 950 ug/g respectively. Kraft and Sypniewski's (1979) contaminated area averaged 589 ug/g copper. Thus the failure to find decreases in benthos density in areas ranging from only 0.6 to 116.4 ug/g Zn, 0.002 to 0.770 ug/g Cd, and 0.76 to 33.40 ug/g Pb may not be surprising. It certainly should not be construed to refute the other studies.

Fish Species Diversity

Fish species diversity reacted in a manner directly opposed to what was anticipated. Instead of decreasing at highway sites and the more contaminated sites (sensu Haedrich 1975), diversity was greater. On the I-295 streams, fish diversity was significantly higher at highway sites on the last three trips. Diversity at downstream sites was never significantly different than that found at upstream sites. There was a quasi-significant trend in fish diversity at highway sites relative to upstream sites, however it was in an unexpected direction, towards increasing diversity at highway sites.

Rather than accept the views of other researchers (e.g. Moore 1979, Kraft and Sypniewski 1981; Chadwick and Canton 1984) that species diversity indices are insensitive to metals pollution, and simply dismiss the results of this study as anomalous, an alternative explanation was sought. I considered what happened in the context of the intermediate disturbance hypothesis of species diversity. The intermediate disturbance hypothesis states that species diversity is greatest at an intermediate level of disturbance, because the disturbance prevents any one species from becomming dominant and thus competitively excluding other species. Connell (1978) showed that species

diversity of tropical rain forests and coral reefs was greatest under conditions of intermediate climatological disturbance (forest fires and hurricanes, respectively). Also, Hixon and Brostoff (1983) found that diversity of algae was greatest under intermediate levels of predation. If heavy metals act as an agent of intermediate disturbance, then it may not be suprising that species diversity does not always decrease when metals levels increase.

Although the intermediate disturbance hypothesis predicts a bell-shaped response to a perturbant, these data show only the ascending part of that curve. This is probably because observed metals levels were not sufficient to eliminate any species and thus cause species diversity to decline. However, the ability of heavy metals to kill various species of fish is well documented, and not in question. As one and then another fish species in a system succumbs to metals pollution, diversity must inevitably decline. Decreasing fish community diversity that may represent the descending arm of the intermediate disturbance curve has been demonstrated by other researchers (e.g. Bechtel and Copeland 1970; Copeland and Bechtel 1971; Haedrich 1975; Tsai 1968).

These results are consistent with a number of current diversity hypotheses that are related to the idea that any mortality applied to a community will tend to reduce

competitive exclusion and thus increase diversity. These include Hutchinson (1948), who restricted it to density independent mortality, the "predation hypothesis" of Paine (1966), the intermediate disturbance hypothesis of Connell (1978), and the dynamic equilibrium "general hypothesis" of Huston (1979) which subsumes all of the above.

An alternate explanation for the higher fish diversity at highway sites is simply that the habitat there is more diverse or stable, and thus capable of supporting more species. If this were the case, however, one would not expect to see the increase in diversity through time that was found at these sites, but rather a steadily higher level, which was not found.

Fish species diversity in the streams crossed by the more heavily travelled I-95 was dramatically lower than those of the I-295 streams. The mean value for diversity at the I-95 highway sites was only 51% of that at the I-295 highway sites over the course of the study. The diversity values for three of the four I-95 highway sites were lower than any recorded for the I-295 streams. These findings are more in line with the traditional views of the relationship between pollution and diversity. However, the extent to which the diversity is lower is probably not completely explainable in terms of the sediment metals levels as recorded in this

study, since (at least on the days sampled) zinc and cadmium levels were about equivalent to, and lead levels only twice as great as those found for the I-295 streams. As previously discussed, these sediment metals levels may be the result of heavy rainfall occurring shortly before the sampling trip. The concentrations of cadmium and lead in fish whole bodies and tissues are certainly indicative of the greater degree of contamination realized at the I-95 streams.

Fish Community Density

Fish density was included in this study because it was reasoned that if toxicity was occurring at the study sites, it might well be reflected in lower numbers of fish at these sites. The very term "toxic pollution" conjures up visions of fishless sections of stream below sources of toxic input. Leland and McNurney (1974) reported an almost complete absence of fish in a metals-polluted Illinois river. However, there were dissolved oxygen deficits in the river as well. Roline and Boehmke (1981) found reduced trout populations in the Upper Arkansas River, Colorado, below inputs of heavy metals.

With respect to the I-295 hypothesis testing section, fish density averaged higher at upstream sites. However, site type differences were, with one exception,

statistically insignificant. There were no significant trends towards decreased fish density at either highway or downstream sites. Fish density varied among streams and seasonally.

The lack of significant findings for fish density is most probably due to the fact that none existed. The statistical tests for biotic parameters should have been quite powerful. Apparently higher metals levels than those encountered in the I-295 streams is necessary before the total number of fishes in the community declines.

Examination of the relationships between sediment metals and fish density confirm the lack of effects of the observed metals contamination on fish densities. There were no significant correlations between the levels of any metal and fish density. The scatter plots do not indicate any type of intelligible relationship. Fish density in these streams does not appear to be normally distributed about a single mean value. I think the biggest source of variation in fish densities in these streams was interstream variation in physical habitat, and not metals contamination.

Evidence for the effects of higher levels of metals contamination over longer periods may come from the fish densities on the I-95 streams. Mean fish density at I-95 highway sites was 70%, 69%, and 57% of that found at I-295

highway, downstream, and upstream sites respectively. Fish density was extremely depressed on Mechump's Creek, being only 0.86 fish per 10m stream. This was in spite of the fact that it was the size of the average I-295 stream (ruling out size differences), and contained good physical habitat.

Fish Community Biomass

The inclusion of fish biomass in the study was a logical extension of the inclusion of fish density. The two variables were expected to parallel each other rather closely, unless changes in the fish community were to occur. While it might be possible for a change to occur without a change in either biomass or density, the chances of it escaping both seemed rather small.

No significant site type differences were found for the I-295 streams over the course of the study, with the exception that fish biomass was significantly lower at the downstream sites relative to the upstream sites on Trip 5. No general temporal trends were found in the fish biomass H/U and D/U ratios. Fish biomass did exhibit inter-stream and seasonal differences.

As with fish density, the lack of significant changes in fish biomass is most likely related to the fact that metals contamination was not severe enough to cause them. Even the

most contaminated of the highway sites (HO, NA) were not depressed in fish biomass.

Examination of the relationships between sediment metals levels and fish biomass offers little to suggest that metals contamination was causing any changes in fish community biomass. There were significant positive correlations between sediment cadmium and lead levels and fish biomass. This would mean that as metals increased, so did fish biomass. However, correlation coefficients were low (about 0.33 for both). I suggest that something else is varying here, with which both fish biomass and sediment metals co-vary in a logical manner. This could perhaps be stream size, or the presence of large pools, in which large fish could live and metals-bearing particulates settle out. The fact that current velocities were low at the more contaminated highway sites is consistent with the presence of more pool-like conditions.

As usual, the I-95 streams show differences in fish biomass that may be due to their greater degree of metals contamination. Fish biomass at I-95 highway sites averaged 27% and 21% of that found at I-295 highway and upstream sites respectively. In agreement with the fish density findings, very low fish biomass was found at the highway site on Mechump's Creek. Even lower biomass was found at

MM94.5 Creek. However, this stream was smaller, about the size of the smallest I-295 streams. Given that, the low biomass there is not too unreasonable.

Fish Community Similarity

Biological integrity is the maintenence of community structure and function that is characteristic of a particular area (Cairns 1977). One of the contributors to biological integrity is inertia, which has been defined as the ability of an ecosystem to resist changes in structural and functional characteristics (Cairns 1977). When external inputs to an ecosystem exceed its inertial capacity, either the ecosystem's structure or function, or both, change.

A similarity index was used in this study to ascertain whether fish community structure at the I-295 sites was changing over time. I found that the similarity of fish community structure on subsequent trips with respect to that present on the first trip at each site was lowest for highway sites, and it decreased through time. Upstream sites exhibited the greatest similarity to original community structure and the structure persisted for the length of the study. Downstream sites had coefficients of similarity intermediate between upstream and highway sites. After two years, community structure was the most changed at

highway sites, and the least changed at upstream sites, with the downstream sites changed an intermediate amount, on every stream. The comparison of fish community structure at highway sites with that at upstream sites over the same interval confirmed that the changes noted at highway sites actually reflected decreased biological integrity at those sites, as opposed to recovery from any construction impact.

The increasing dissimilarity of fish community structure with respect to original conditions at highway and downstream sites is exactly what was expected to occur, if the highway was having effects on the fish communities of roadside streams. Effects were greatest at highway sites, intermediate at downstream sites, and not found at upstream sites. Although the similarity of upstream sites is about 0.8 instead of 1.0, it cannot be expected that the species and numbers of fish per species present would be exactly the same every year. The fact that the upstream fish community similarity values did not vary over time, is what is important.

These results are in agreement with the changes found in fish diversity at the study sites. In fact, the two indices are related in that they both use some of the same information (i.e. the numbers of species present, and the numbers of individual of individuals present per species).

The similarity index uses additional information, the actual identity of the species present. This overcomes one of the criticisms of diversity indices, namely that fish community changes that represent the replacement of one species by another (or replacement of all species, for that matter) would not be detected (Washington 1984). In that respect, the similarity index is more powerful in detecting community changes than are diversity indices. Proof that they are is not provided by this study. Both indices detected spatial and temporal changes in community structure.

CONCLUSIONS

I investigated the contamination by and effects of highway-generated heavy metals in six roadside stream ecosystems along a new highway. An experimental approach was employed that allowed assessment of both spatial and temporal changes in metals concentrations and biotic parameters. I found that significant metals contamination occurred at sites located directly at the highway and, to a lesser degree, sites located 500m downstream of the highway. Much variability existed in the extent to which the different streams became contaminated. Most of the variability could be explained by differences in physical features among the streams and proximity to the highway surface. Statistical elucidation of temporal trends in metals contamination was hampered by the fact that contamination plateaued or reached an equilibrium after about one year of highway usage, rather than increasing continually.

Three of the seven biotic parameters investigated showed changes reflecting the differential metals contamination of the sites. The percentage of the aquatic insect community

that was composed of chironomids increased with increasing sediment metals contamination. Fish community species diversity increased at highway sites through time. The similarity of fish community structure at the study sites through time was greatest for sites located upstream of the highway, least at highway sites, and intermediate at sites located downstream of the highway, indicating that biological integrity at highway and downstream sites was disrupted.

As originally conceived, there were four possible scenarios regarding metals contamination and the subsequent response of the biotic parameters regarding the effects of Interstate 295. The first was that there would be no increase in metals and no changes in biotic parameters over time. This would have indicated that contamination had not occurred. The second was that metals did increase but no changes in biotic parameters were observed. In this case, although contamination had occurred, it was insufficient to cause discernible effects. The third possible outcome was that there was no increase in metals, but the biotic parameters did change. This could be attributed to other unmeasured stressors, or a highly dynamic and variable ecosystem. The final possibility was that metals levels increased, and biotic parameters changed. A reasonable

explanation here, in the lack of alternate explanations, is that metals are causing the changes.

Perhaps if only one metal and one biotic parameter on one stream had been measured, the outcome might have been as simple as originally thought. As it turned out, the four scenarios were not mutually exclusive. All four were true with respect to at least one element of the study. However, enough consistency was apparent to reach the following general conclusion about the effects of highway usage on the I-295 streams. Metals levels in most ecosystem components at highway, and, to a lesser degree, downstream sites were increased moderately over background levels, but sufficiently to cause changes in some, but not all, of the biotic parameters employed to assess ecosystem health.

Analysis of Experimental Approach

The metals sampling regime was for the most part adequate to assess statistical differences at an alpha of 0.1. It is recommended that water metals analyses be taken on a larger scale, including sampling during rainfall events, analysis of both total and dissolved metals, and more replicates per site, if more insight into its contamination is desired. The lack of sufficient numbers of the same species of organisms at all sites made testing for differences among

benthos, whole-body fish, and fish tissues more difficult than it desireable. Although statistical procedures were used that compensated for this problem, I strongly recommend that the problem be avoided wherever possible, if for no other reason than it will be much easier to communicate results. A final recommendation (which applies to biotic parameters as well) is that adequate presampling be done to ensure that reference (control-like) areas are reflective of background levels. An added benefit here is that this sampling would also allow an estimate of sample variance, and thus make possible the determination of sample sizes necessary to achieve the desired levels for Type I and Type II error (sensu Cohen 1977; Conquest 1983; Toft and Shea 1983). The use of ratios between parameters at reference and "treatment" sites (Thomas and Eberhart 1976) was a very helpful technique in removing confounding seasonal and site specific effects.

The biological parameters which were measured showed a range of sensitivity to the effects of metals contamination of the roadside streams. Indications of perturbations were found in both benthic invertebrate and fish communities. With respect to benthic invertebrates, the most sensitive measure of the effects of metals contamination was the percentage of the aquatic insect community that was composed

of chironomids, followed by benthos invertebrate density and diversity. Benthic invertebrate diversity may not have shown any changes because of insufficient taxonomic differentiation. Given the much greater time necessary to do this however, it is hard to recommend the use of benthic invertebrate diversity when other parameters (e.g. AIPC) can identify perturbations with much less effort. However, the ability of AIPC to show an increase when the percentage of chironomids is high to start with should be further scrutinized.

Fish community changes due to the low levels of metals contamination experienced were detected by both the Shannon diversity index and Pinkham and Pearson's coefficient of similarity. The results experienced with the Shannon index (significant deviations, but in the "wrong" direction) show that the use of diversity indices should be tempered with an understanding of the underlying principles. The success of the Pinkham and Pearson coefficient of similarity bodes well for its further usage in water quality studies. Fish community density and biomass, although giving indications of responding as expected, are probably useful only in more severely contaminated environments. They were depressed in streams along the more heavily travelled I-95. However, their usage is complicated by differences in physical factors (e.g. size, depth) among streams.

Finally, I suggest that two-sided statistical tests be used in the statistical testing of the response of biotic parameters to environmental contaminants. The unexpected direction of changes in benthic invertebrate density and fish diversity at highway sites over time, suggest that the disallowance of subsidy effects to toxic input by Odum et al. (1979) in their "subsidy-stress gradient", may not be tenable. Low levels of metals contamination may alter biotic parameters in ways usually thought of as beneficial.

The Significance of Highway-generated Heavy Metals

The goal of this study was to determine if presently observed conditions of automotive traffic are causing significant changes in the integrity of roadside stream ecosystems. The traffic densities encountered on I-295 in this study averaged about 12,000 vehicles per day. This density is not especially high in relation to many highways that may average up to 100,000 vehicles per day, or more. In that respect, many other highways would be expected to show greater levels of contamination than found in the I-295 streams.

On the other hand, the study streams had physical and chemical features (e.g. small size, low hardness, low pH) that would tend to make them more susceptible to heavy metal

toxicity than many other streams. Although necessarily subjective, considering traffic density and susceptibility to toxicity, I would characterize the potential for ecological disruptions at the study sites as about average, compared with roadside streams in general.

The nature and magnitude of changes with regard to contamination and effects found at the I-295 highway sites are indicative of low to moderate levels of pollution, as compared to published reports for other sources of pollution. No "fish kills" were observed, and it is doubtful that any metals-related human health problems would arise from the consumption of fish caught at any of the highway locations.

Results of sampling along the older and more heavily travelled I-95 (50,000 vehicles per day) indicate that greater contamination and more pronounced biological effects will be found as a result of higher traffic levels, over a greater period of time. These streams exhibited some of the more classic sequelae of pollution, including reduced fish diversity, density, and biomass, and reduced benthic invertebrate density.

It is thus clear that highways are a potentially significant source of heavy metals pollution to roadside streams. Site-specific factors mediate the actuality of

contamination and effects thereof. Prudence dictates the consideration of the above in the planning of new highways when socially or economically valuable resources are involved.

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APPENDIX TABLES

Appendix Table 1. Fish species collected by stream (A = abundant, C = common, R = rare)[1].

Species	HO	BD	BR	NO	SL	NA
Lampetra aepyptera		C	A	A		
Petromyzon marinus		R				
Amia calva		R				
Anguilla rostrata	C	A	C	C	A	A
Umbra pygmaea	A	R	R	C	A	R
Esox americanus		R	R		R	R
Esox niger		R			R	R
Clinostomus funduloides	R					
Nocomis leptocephalus		R	A		C	
Notemigonus crysoleucas	R	C				R
Rhinichthys atratulus	R	R	C	A	R	R
Semotilus atromaculatus	A		A	A	A	A
Erimyzon oblongus	C	C	R	R	R	A
Ictalurus natalis	R	R	R		R	C
Ictalurus nebulosus	R				R	R
Noturus insignis		R	R		R	R
Aphredoderus sayanus	A	R	R	R	C	A
Chologaster cornuta					R	
Gambusia affinis	R	C			R	R
Centrarchus macropterus		R	R		R	C
Enneacanthus gloriosus		C	R		R	C
Lepomis auritus		R				C
Lepomis gibbosus	C	C			R	C
Lepomis gulosus	R	C			R	R
Lepomis macrochirus	C	A	R		C	R
Micropterus salmoides	R	R			R	
Pomoxis annularis		R				R
Etheostoma olmstedi	R	C	A		C	C

[1] A > 10% ≥ C ≥ 2% > R, where the percentage refers to the total number of fish collected on the stream.

Appendix Table II. Identity of I-95 study streams and the types of samples taken at each.

STREAM	SITES	METALS	BIOTIC
MM94.5 Creek	H	X	X
Mechump's Creek	U	X	X
	H	X	X
Flippen Creek	H	X	X
Kingsland Creek	H	X	
Walthall Stream	U	X	X
	H	X	X

Appendix Table III. Instrumental parameters for Perkin-Elmer model 460 spectrophotmeter. Air-acetylene flame operation (from Martin 1983).

Parameter	Cadmium	Lead	Zinc
Wavelength (nm)	228.8	217.0	213.9
EDL Lamp Power (watts)	5	10	6
Detection Limit ug/ml	0.02	0.10	0.05
ug/g[1]	0.10	0.50	0.25

[1]Based on sample dry weight of 0.6 g dissolved in 3 ml HNO3.

Appendix Table IV. Instrumental parameters for Perkin-Elmer model 460 spectrophotometer with HGA 2100 graphite furnace (from Martin 1983).

Parameter	Cadmium	Lead
Wavelength (nm)	228.8	217.0
EDL Lamp Power (watts)	5	10
Purge Gas	Ar	Ar
Gas Flow	Normal	Interrupt
Drying Time (s)	28	28
Drying Temperature (C)	50	100
Charring Time (s)	28	28
Charring Temperature (C)	250	500
Atomization Time (s)	9	9
Atomization Temperature (C)	2100	2100
Detection Limit		
ng/ml	0.5	2
ng/g[1]	20	80

[1]Based on average sample dry weight of 0.1 g dissolved in 4 ml HNO3.

Appendix Table V. National Bureau of Standards Reference Material 1577 (bovine liver) certified concentrations versus concentrations determined at V.P.I. & S.U. Department of Fisheries and Wildlife Sciences. Flame atomic absorption spectroscopy conditions. N = 8. All values ug/g, d.w. (from Martin 1983).

	Cadmium	Lead	Zinc
Certified Concentration			
mean ± S.E.	0.44±0.06	0.135±0.015	123±8
Obtained Concentration			
mean ± S.E.	0.43±0.01	bd[1]	111±14

[1] Below the limit of detection.

Appendix Table VI. National Bureau of Standards Reference Material 1577 (bovine liver) certified concentrations versus concentrations determined at V.P.I. & S.U. Department of Fisheries and Wildlife Sciences. Graphite furnace conditions. N = 8. Values ug/g, d.w. (from Martin 1983).

	Cadmium	Lead
Certified Concentration		
mean ± S.E.	0.44±0.06	0.135±0.015
Obtained Concentration		
mean ± S.E.	0.43±0.07	0.138±0.006

Appendix Table VII. Recoveries (%) based on spiked samples. Mean ± S.E.; N=10 pools for each metal and sample type under flame atomic absorption spectroscopy conditions.

Sample	Zinc	Cadmium	Lead
Sediment	98.2±3.0	99.8±3.3	97.8±1.5
Benthos	110.4±2.0	--	--
Whole-body	103.5±4.8	91.6± 2.9	108.0±7.7
Liver	110.5±7.1	109.7±16.5	95.1±5.6
Kidney	94.1±6.5	96.2± 7.8	94.4±7.7
Bone	94.7 5.8	--	97.5±2.5

Appendix Table VIII. Recoveries (%) based on spiked samples. Mean ± S.E.; N = 10 pools for each metal and sample type under graphite furnace conditions.

Sample	Cadmium	Lead
Sediment	96.0±4.3	95.6±4.9
Benthos	98.8±5.2	104.2±2.3
Liver	96.3±8.3	97.0±1.2
Kidney	110.4±3.2	97.7±4.3
Bone	--	107.1±4.2

VITA

John M. Mudre was born on February 12, 1953, in Washington D.C. He graduated from Washington-Lee High School, Arlington, Virginia, in June, 1971. He then attended Virginia Polytechnic Institute and State University, graduating with a Bachelor of Science Degree in Forestry and Wildlife Sciences, Fisheries Option, In December, 1975. Following that, he entered the graduate program in Fisheries at the University of Georgia, Athens, Georgia, graduating with a Master of Science Degree in Forest Resources-Fisheries in March, 1979.

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