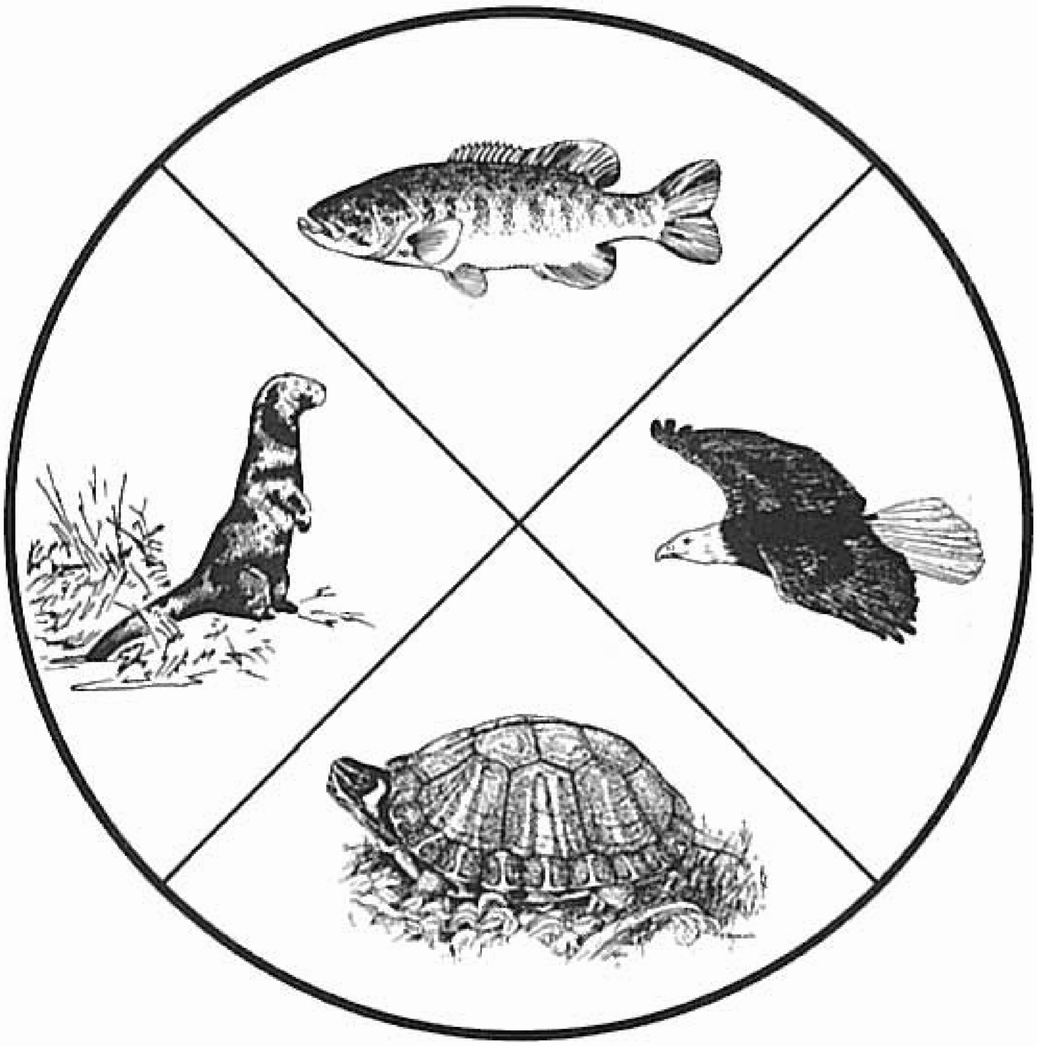
FORT WORTH FIELD OFFICE
ECOLOGICAL SERVICES

CONTAMINANTS REPORT
NOVEMBER 1988

IMPACTS OF TOXIC CHEMICALS ON TRINITY RIVER FISH AND WILDLIFE



FISH AND WILDLIFE SERVICE

U.S. DEPARTMENT OF THE INTERIOR

IMPACTS OF TOXIC CHEMICALS ON TRINITY RIVER FISH AND WILDLIFE

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EXECUTIVE SUMMARY

Prior to 1850, native Americans, early settlers, and fish and wildlife were attracted to the upper Trinity River of Texas as a source of good water in a relatively dry land. As the populations of Fort Worth and Dallas boomed, the Trinity became a convenient place to dump sewage and industrial wastes. By 1925 the small river was losing the battle with the growing amount of wastes from the cities and reminded observers of "a mythological river of death."

Since passage of the 1972 Clean Water Act, most industrial and small municipal discharges in the Dallas/Fort Worth area have been diverted to large, regional sewage treatment plants rather than discharging directly to the river, and the condition of the river has improved somewhat. However, as the population of the Dallas/Fort Worth urban area now approaches four million people, urban runoff impacts have increased, sewage treatment plants have been hard pressed to keep up with population and industrial growth, and fish kills have continued to occur on an intermittent basis downstream of Dallas. The regulatory revisions introduced by the Water Quality Act of 1987 have created a new emphasis on identifying and correcting water quality problems caused by urban runoff and other non-point source discharges.

In anticipation of these information needs, collections for this study, the first large scale study of contaminants in Trinity River aquatic life, were initiated in mid-July, 1985, just after the first large fish kill of the summer.

Key objectives of the study included identifying: 1) which fish and wildlife species in the Trinity River are accumulating potentially harmful body burdens of toxic contaminants, 2) locations of chemical "hot spots," 3) contaminants whose presence is correlated with industrial, municipal, illegal, or residential runoff, 4) initial estimates of the impacts of various types of runoff on fish populations, 5) potential impacts of individual toxic chemicals on Trinity River fish and wildlife, and 6) contaminant information providing insight into the potential causes of Trinity River fish kills.

Residues of 67 chemical contaminants were measured in tissues of up to 17 species of fish, turtles, clams, and crayfish from 27 Trinity River sites. All species were not present at every site, and cost limitations prevented our examining every sample for every contaminant. A total of 64 samples were analyzed for organochlorines and polychlorinated biphenyls (PCBs), 77 samples were analyzed for heavy metals, 33 samples were analyzed for polycyclic aromatic hydrocarbons (PAHs), and 6 samples were analyzed for aliphatic hydrocarbons. The collection sites extended from Mustang Creek above Fort Worth through Dallas to the Highway 79 area approximately 250 river miles downstream.

The contaminants found to be most consistently elevated in fish and wildlife of the upper Trinity River were PCBs, total(combined) chlordane, total non-DDT organochlorine pesticides, lead, and mercury. The concentrations of these contaminants were high enough to warrant concern for predatory species of fish and wildlife in at least one half of all samples. The concentrations of combined heavy metals were also elevated in at least one half of all samples, but more research is required before we can determine the extent to which the recorded levels of combined heavy metals are harmful to various species of fish and wildlife. Several additional contaminants were frequently elevated in tissues of fish and wildlife at sites just downstream of Dallas (between South Loop 12 and Malloy Road Bridge). Contaminants in this category included dieldrin, total polycyclic aromatic hydrocarbons (PAHs), and chromium. Concentrations of these contaminants were high enough to warrant concern for predatory species of fish and wildlife in at least one third of all samples collected at the sites just downstream of Dallas.

The potential impact modes of some of the contaminants detected in Trinity River fish and wildlife are not confined to direct toxicity. For example, several of the PAHs and other contaminants detected have been documented to be carcinogenic (producing or inciting cancer), tumorigenic (producing or tending to produce tumors), teratogenic (inducing deformities in a fetus or embryo as it develops), and mutagenic (inducing genetic mutations) to certain species of fish or wildlife. A summary of what we know about effects harmful to fish and wildlife is included in text discussions of each contaminant.

To obtain an understanding of the uniformity of contamination of fish and wildlife tissues in the impacted area downstream of Dallas, a detailed, multi-species comparison was made between a site there and a presumably cleaner, control/reference site. The site chosen as the control/reference site was Mustang Creek, a tributary to Lake Benbrook in the Trinity River headwaters southwest of Fort Worth. The Mustang Creek site is upstream of known pollution sources in Dallas/Fort Worth. Except for zinc, manganese, and heptachlor epoxide, residues of all 67 contaminants were higher in indicator tissues of six species of fish and turtles from the site downstream of Dallas (Trinity River at Highway I-20/635 bridge) than from those collected at the Mustang Creek site.

Other "hot spots" for contaminants in the upper Trinity River included downtown storm drain sites. For example, the concentration of total PCBs in a whole-body composite sample of spiny softshell turtles from a storm drain canal in downtown Fort Worth was 20 mg/kg, a highly elevated level. These turtles were collected from the only site on the Trinity where we have observed anyone fishing specifically for turtles.

However, the focus of this study is effects of contaminants on fish and wildlife rather than effects on humans. Predatory species of fish and wildlife are often more at risk from consumption of contaminated fish and wildlife than are humans. This is because fish and wildlife typically account for a larger percentage of their diet and because predatory species typically consume the whole body of prey rather than a fillet portion (see PCB section for a more detailed discussion of risk factors).

Gradient monitoring analysis revealed that mercury and aluminum concentrations in mosquitofish had positive statistical correlations with presence of treated, chlorinated sewage. A separate statistical analysis confirmed that aluminum levels in mosquitofish from sites having substantial treated sewage were significantly higher than levels in mosquitofish from sites having little or no treated sewage. Lindane was detected in fish and wildlife only at sites downstream of sewage plants.

At mainstem sites where there was little or no influence of raw sewage, mosquitofish had significantly lower concentrations of mercury than mosquitofish from other sites. Lead concentrations in mosquitofish from all sites were closely correlated ($P<0.02$) with presence of raw sewage.

Mosquitofish body burdens of iron, dieldrin, combined chlordane, all chlordane components, aluminum, and lead were significantly lower at sites having little or no residential runoff than at other sites. Correlation coefficients between residential runoff and contaminants were most significant ($P<0.02$) for oxychlordane, *trans*–nonachlor, *cis*–nonachlor, combined chlordane, and dieldrin.

PCBs, lead, chromium, and iron levels in mosquitofish were significantly lower at sites where there was little or no influence of industrial runoff than at other sites. Of these variables, PCBs had the most significant statistical correlation with presence of industrial runoff. Lead and PCBs were significantly lower in mosquitofish from sites having little or no runoff from downtown areas than those from other sites.

Our findings suggest that not all of the contaminant problems in the Trinity originate in sewage treatment plant discharges and legally-permitted industrial discharges. Illegal connections of raw sewage and industrial waste lines to storm drains, overloaded pipelines, and pipeline leaks result in frequent discharges of raw sewage and industrial wastes into the Trinity River.

Numerous other sources of toxic chemicals are less obvious but may be significant on a cumulative basis to a small river such as the Trinity. For example, the lack of convenient recycling/disposal centers for used motor oil and other hazardous chemicals is one of many factors which may indirectly encourage their introduction into the river. Many of these hazardous materials currently find their way into the river via storm drains.

Leachates originating from the many landfills adjacent to the river may also be playing an indirect role in transporting toxic chemicals to the Trinity, particularly leachates from illegal landfills and older municipal landfills. More study is required to quantify the relative contributions of toxic chemicals to the Trinity River from the numerous potential sources in the Dallas/Fort Worth area.

An analysis of the number of species of fish and the percentage of pollution–tolerant forms revealed an upstream to downstream degradation of fish populations as the river moved through Fort Worth and Dallas. Impacts on populations of small fish progressed from 1) little or no impact at Mustang Creek upstream of Fort Worth, to 2) some impacts in downtown Fort Worth, 3) greater impacts downstream of downtown Fort Worth, 4) severe impacts from just downstream of the first large sewage treatment plant (Fort Worth's Village Creek Plant) to an area well south of Dallas, and 5) some (but not total) recovery 250 miles downstream.

Sites with major presence of raw or treated sewage had greater impacts on fish populations, including fewer species present and a greater percentage of pollution–tolerant species, than sites having little or no sewage or an intermediate amount. The degree of impact to fish populations correlated better with the presence of raw or treated sewage than with the presence of low dissolved oxygen.

There was a statistically significant, positive correlation between the sum of residential runoff, industrial runoff, downtown runoff, presence of raw sewage, and presence of chlorinated sewage with impacts on populations of small fish. This finding supports the theory that cumulative stresses from different types of runoff may be impacting fish populations.

A cumulative impact variable which correlated even more closely with overall impacts on fish populations was created by giving double weighting to presence of chlorinated, treated sewage; double weighting to chlorine–adjusted raw sewage; single weighting to presence of industrial runoff; and no weighting to residential runoff, downtown runoff, or the presence of low dissolved oxygen.

Since these results suggested chlorine may be a significant factor affecting fish distribution, follow-up toxicity tests were conducted in the river downstream from chlorinated discharges. Initial tests revealed that on some occasions there was instream toxicity to fish as far as five miles downstream from the sewage discharge, a rapid kill of test fish near the discharge point, and a lack of the legally required non-toxic zone for migration of fish. These tests on Trinity River discharges confirmed results previously documented in the literature for chlorine impacts at other sites.

We believe that strict limitations on discharges of chlorine, a change currently being planned for sewage treatment plants large enough to be considered major dischargers, may significantly help fish and wildlife populations downstream of these plants. We recommend that the chlorine limitations be extended to point source discharges of all sizes.

In summary, many species of aquatic fish and wildlife which would occur in the Trinity River downstream of Dallas/Fort Worth, if the river were healthy, were absent from the area. Many of the species present were found to be carrying high enough body burdens of toxic chemicals to cause concern for their welfare and for predatory species of fish and wildlife consuming them. Potential sources of these chemicals, study needs, and possible remedial actions are discussed.

Keywords - Urban Runoff Residues PCBs Chlordane Chlorine Sewage Pesticides Lead Mercury Dieldrin Polycyclic Aromatic Hydrocarbons (PAHs) Chromium Lindane Aluminum

INTRODUCTION

Early settlers' descriptions of the Trinity River at Fort Worth, Texas, were laudatory, and the river probably had particular value for fish and wildlife to the west of Dallas, where aquatic resources were rare prior to the creation of today's man-made impoundments. Although the upper reaches of such streams are intermittently dry, some pools usually persist. The pools provide a source of water for wildlife and remnant populations of fish which can join with downstream populations to recolonize the river when the water rises. As late as 1886, scientists collected fish species which are intolerant of pollution from the Trinity River at Dallas [1].

As Fort Worth and Dallas boomed, the Trinity River was used as a convenient place to dump sewage and industrial wastes and by 1925 the urban section of the river was described as an "inky surface" which brought to mind "a mythological river of death" [2]. The pollution impact was exacerbated by the fact that the Trinity is a small river, so its ability to harmlessly assimilate contaminants is limited. Today, the Trinity has more industrial development and a greater human population than any other river basin in Texas. The Dallas/Fort Worth area is the largest population center in the country that is not located close to the Great Lakes or ocean shores (Sam Brush, North Central Texas Council of Governments, personal communication).

Since passage of the 1972 Clean Water Act, most industrial and municipal discharges in the Dallas/Fort Worth area have been routed to large, regional sewage treatment plants rather than connecting directly to the river. Downtown Fort Worth is upstream of any discharges from large sewage treatment plants, and water quality there has improved enough to allow for a wintertime trout fishery. Also, the highly polluted zone of the river south of Dallas is now shorter. However, water quality problems [3,74] and large fish kills, including massive kills in 1984 and 1985, have continued to occur in the river below Dallas.

Although the quality of the waste water being discharged by industrial and municipal point (single pipe) sources has been improved, the quantity of waste water is now much greater due to population growth in the Dallas/Fort Worth urban area. During low flow conditions, treated sewage and illegal discharges now make up a much larger percentage of the flow of the river, so the big city/small river confrontation continues.

As increasingly costly progress continues to be made in improving the quality of direct discharges into the river, more attention is being turned to indirect discharges such as urban runoff and illegal discharges. The passage of the Water Quality Act of 1987 brings a new emphasis on identifying and correcting water quality problems caused by urban runoff and other non-point source discharges.

In anticipation of these information needs, collections for the current study were initiated in mid-July, 1985, just after the first large fish kill of the summer.

A key objective of the study was to determine whether fish and wildlife resources in the Trinity River are accumulating potentially harmful body burdens of toxic contaminants and, if so, to obtain a preliminary indication of whether industrial, municipal, illegal, or residential sources appear to be the probable origin of those contaminants. The study was also designed to identify locations where fish and wildlife are carrying high concentrations of toxic chemicals, to assess the utility of using mosquitofish for monitoring gradients of chemicals, and to document any contaminant data which might contribute insight into the causes of Trinity River fish kills.

MATERIALS AND METHODS

Study Design and Sample Collections

Mosquitofish (*Gambusia affinis*) were chosen as an overall indicator to study body burdens of contaminants versus river mile locations, to identify chemical hot spots, and to identify locations of potential contaminant sources. One large (N>100) sample of mosquitofish was collected for contaminant analysis at each of 24 Trinity River collecting sites (Figs. 1,2; Appendix 1 for location details). Sites were grouped by location type to provide replicate statistical comparisons of different types of sites.

Mosquitofish are very pollution-tolerant and were the only species available at all sites. Another factor favoring use of mosquitofish was that in previous studies [4], mosquitofish became resistant to certain chemicals, allowing

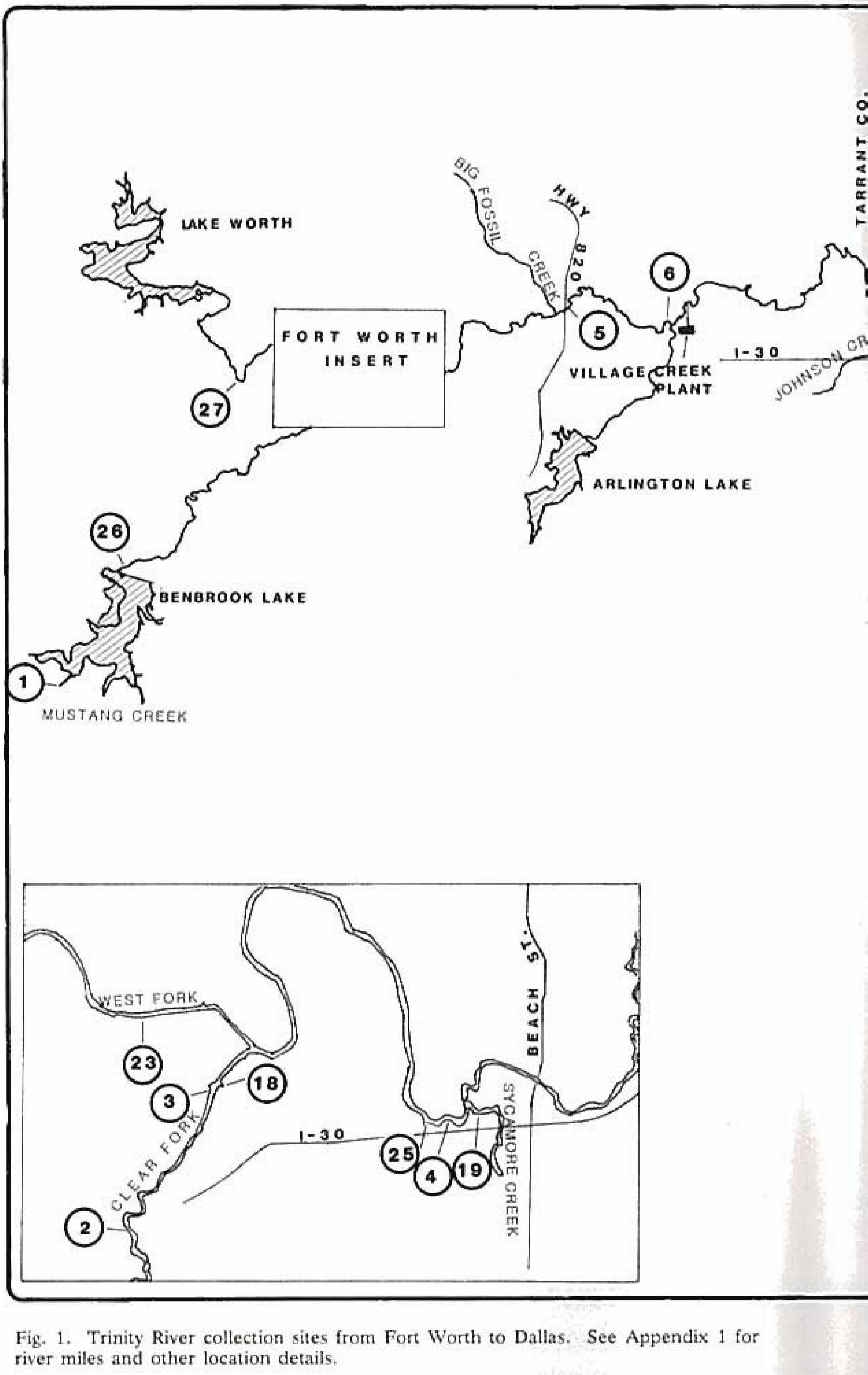
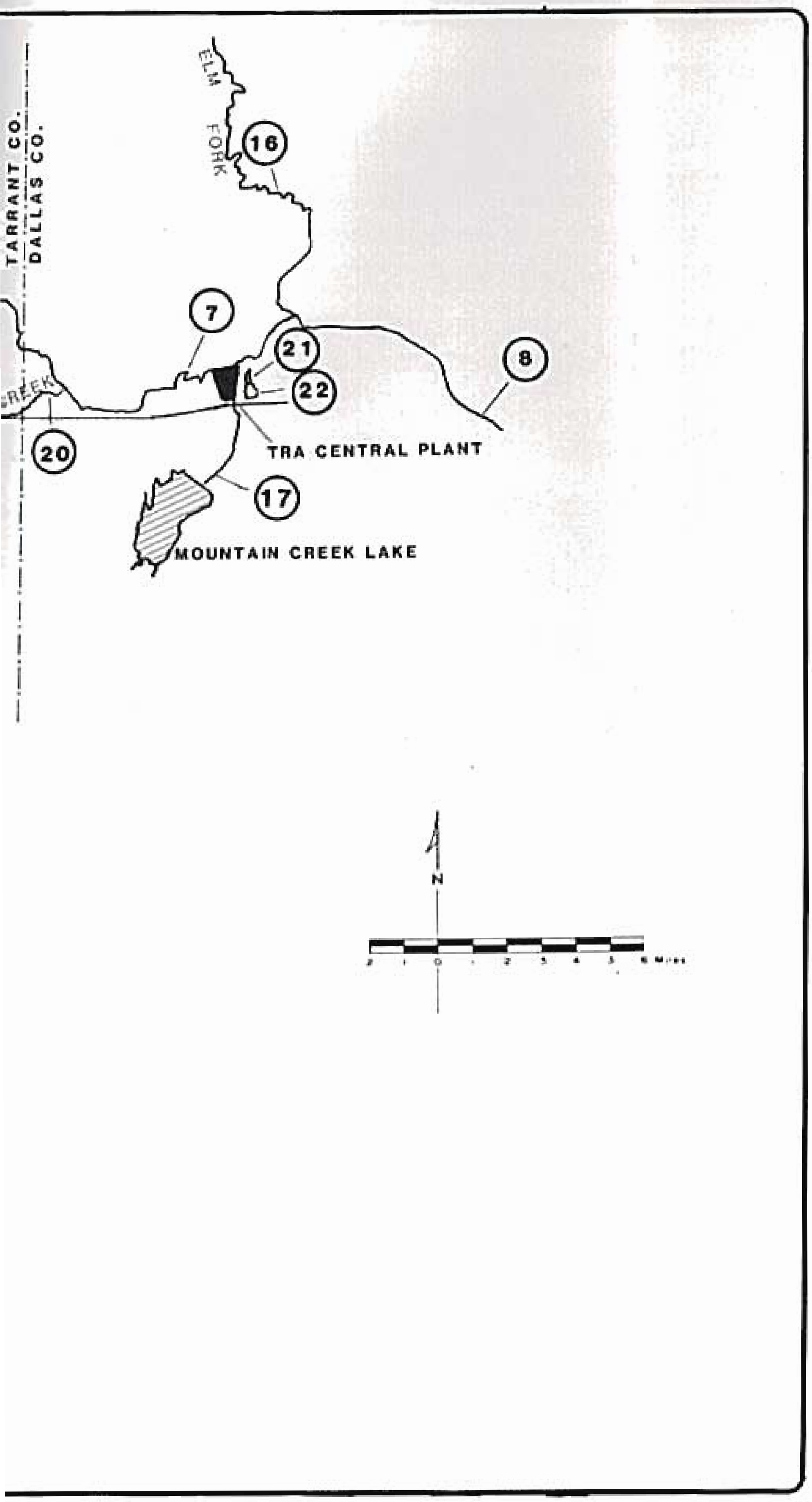


Fig. 1. Trinity River collection sites from Fort Worth to Dallas. See Appendix 1 for river miles and other location details.



LEGEND

SAMPLING	SITES
(1) MC	1
(2) VSB	2
(3) OOD	3
(4) RDB	4
(5) 820B	5
(6) WOVC	6
(7) GH	7
(8) CCC	8
(16) ELF	16
(18) IOD	18
(19) SC	19
(20) JC	20
(21) MP	21
(22) MS	22
(23) UD	23
(25) RSB	25
(26) GC	26
(27) WF	27
(17) MTC	17

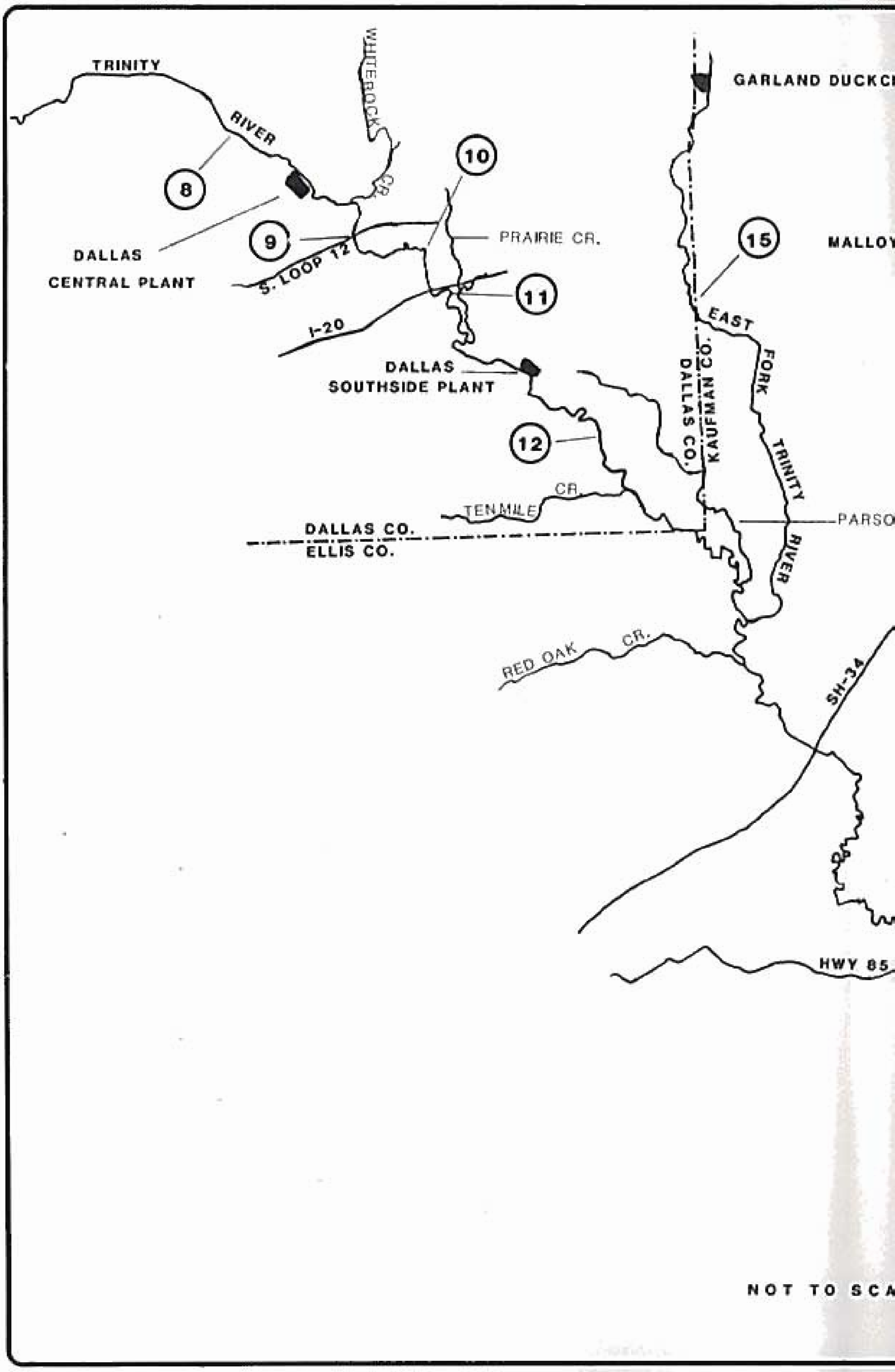
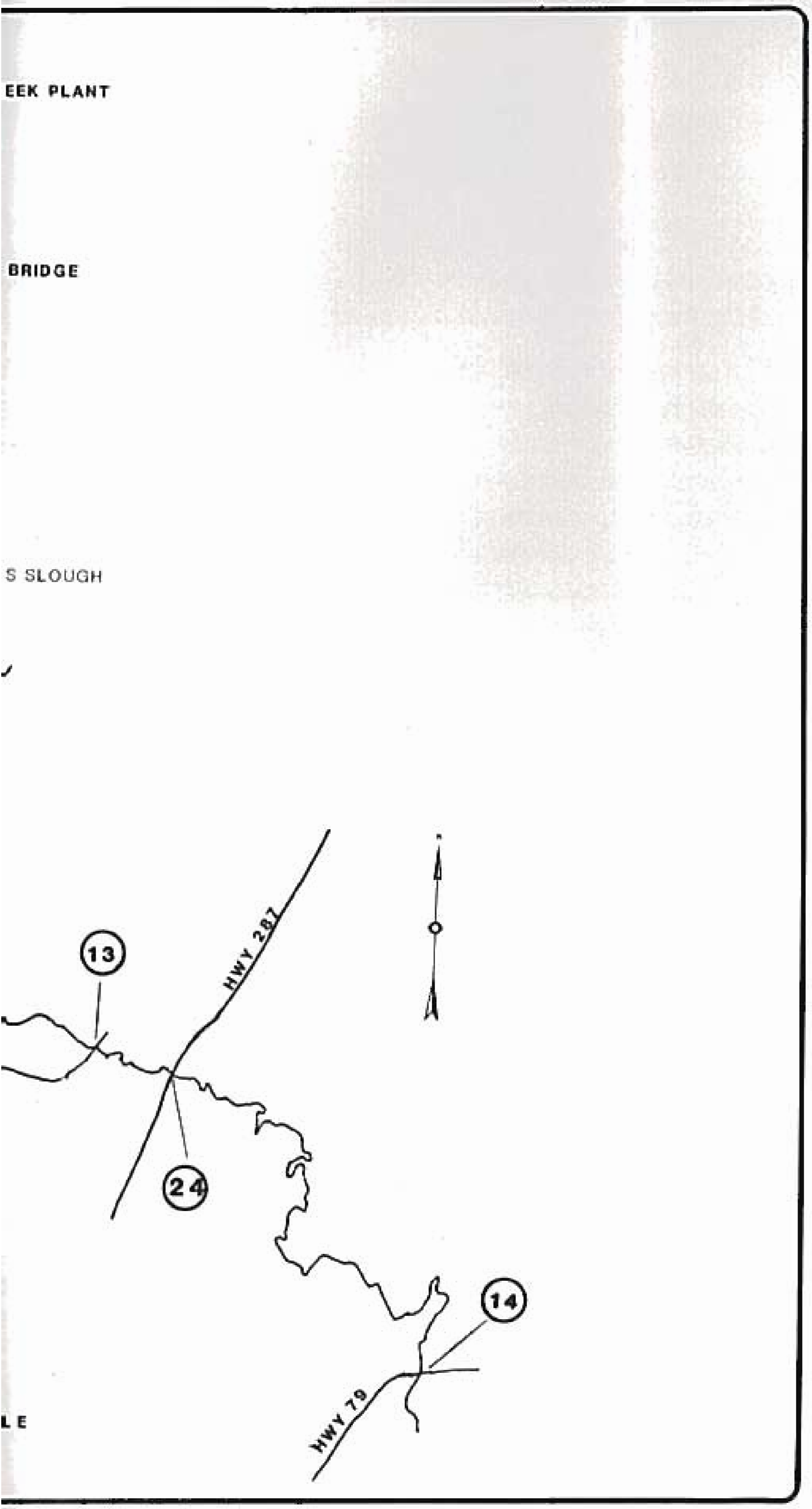


Fig. 2. Trinity River collection sites from Dallas to Highway 79 near Oakwood. See Appendix 1 for river miles and other location details.



LEGEND

SAMPLING		SITES
(8)	CCC	8
(9)	12B	9
(10)	5W	10
(11)	635B	11
(12)	MB	12
(13)	85B	13
(14)	TB	14
(15)	EAF	15
(24)	287	24

elevated body burdens to build up and thereby becoming a dietary hazard to predatory species.

All collections of small fish were made with a 3.66 m long minnow seine with a 0.476 cm mesh. Other fish species collected, while doing equal-effort seining for mosquitofish, were identified to obtain the number of small fish species collected at each site. The percentage of species collected which are considered to be pollution-tolerant was also noted.

To obtain a multi-species, multi-contaminant statistical comparison of overall bioaccumulation differences between a presumably clean (control/reference) site and a site known to be highly impacted by contaminants, intensive collections were made at Site 1 (Mustang Creek) above Benbrook Lake and at Site 11 (I-635) below Dallas. Using seines, gill nets, and hoop nets, the following species were collected at each site: carp (*Cyprinus carpio*), mosquitofish (*Gambusia affinis*), red-eared slider turtles (*Trachemys scripta*), spiny softshell turtles (*Trionyx spiniferus*), bullhead minnows (*Pimephales vigilax*), and smallmouth buffalo fish (*Ictiobus bubalus*). Except for smallmouth buffalo fish, for which only one specimen could be collected at Mustang Creek, all samples were composites of at least 3 specimens each.

A few other species of fish and wildlife were collected for anecdotal and comparative data in spite of the fact that we could find them only at a few sites. These additional collections provided a more complete understanding of the range of body burden concentrations which might be found in other species of fish and wildlife. Included were Asian clams (*Corbicula fluminea*), Mississippi map turtles (*Graptemys kohnii*), common snapping turtles (*Chelydra serpentina*), the Texas cooter turtle (*Pseudemys concinna texana*), channel catfish (*Ictalurus punctatus*), freshwater drum (*Aplodinotus grunniens*) , Unionid clams (*Lampsilis anadontoides*), crayfish (*Procambarus sp.*), blue catfish (*Ictalurus furcatus*), longnose gar (*Lepisosteus osseus*), and redfin shiners (*Notropis umbratilis*).

Laboratory Methods

Tissue analyses for the organochlorine and metal contaminants listed in Table 1 were conducted at laboratories under contract to the U.S. Fish and Wildlife Service. All tissue concentrations in this report are stated as mg/kg (parts per million) wet weight unless otherwise identified.

A graphite furnace technique was utilized for aluminum, cadmium, lead, nickel, and chromium. Mercury was determined with a cold vapor atomic absorption spectrophotometer. A hydride generation atomic absorption spectrophotometer was used for arsenic and selenium and all other metals were detected with an inductively coupled plasma atomic emission spectrophotometer (ICP).

Chemical analysis for organochlorines in tissues was accomplished by gas-liquid chromatography after extraction, gel permeation chromatography cleanup, and silica gel chromatography separation. Ten percent of the reported results were confirmed by gas chromatography-mass spectrometry.

The Mississippi State Chemical Laboratory at Mississippi State University performed the analyses for organics and the Environmental Trace Substances Research Center in Columbia, Missouri, performed the analyses for metals. These laboratories were subjected to a rigorous evaluation process prior to the award of their contracts. The Patuxent Analytical Control Facility of the U.S. Fish and Wildlife Service closely monitored the performance of these laboratories during the analyses and has confidence in the accuracy of the data. Acceptable performance (recovery variation <20% for all chemicals detected) on spikes, blanks, and duplicates was documented in laboratory quality control reports.

Table 1. Organochlorine and Metals Analyses, Trinity River, Texas, 1985

Organochlorine scan:

Hexachlorobenzene (HCB)	Chlordane related:
Hexachlorocyclohexane (BHC)	*cis*-Chlordane (alpha)
alpha-BHC	*trans*-Chlordane (gamma)
gamma-BHC (Lindane)	Oxychlordane
beta-BHC	*cis*-Nonachlor
delta-BHC	*trans* -Nonachlor
Endo Sulfate	Heptachlor Epoxide
Toxaphene	
PCBs (total)	
Dieldrin	o, p'-DDE
Endrin	p, p'-DDE
Mirex	o, p'-DDD
Dacthal	o, p'-DDT
Endosulfan I	p, p'-DDD
Endosulfan II	p, p'-DDT

Metals:

Aluminum (Al)	Lead (Pb)
Arsenic (As)	Manganese (Mn)
Beryllium (Be)	Mercury (Hg)
Cadmium (Cd)	Nickel (Ni)
Chromium (Cr)	Selenium (Se)
Copper (Cu)	Thallium (Tl)
Iron (Fe)	Zinc (Zn)

Statistical Methods

A personal computer with Lotus 1-2-3 software was used for data entry and simple plots; a Statgraphics program from STSC, Inc. was utilized for all statistical analyses. All references to "significantly lower" or "significantly higher" in this report refer to the accepted level of statistical significance ($P<0.05$) unless otherwise specified. The differences between independent samples were tested with the Mann-Whitney nonparametric statistical test, while paired data were tested with the Wilcoxon nonparametric procedure.

The statistical convention used to handle values below the detection limit was to transform them to a value half way between zero and the detection limit. This convention is recommended by Fish and Wildlife Service statisticians for studies such as this one, since a "non-detected" observation does not necessarily mean that the chemical was absent (Christine Bunck, U.S. Fish and Wildlife Service, personal communication). A non-detected observation signifies that the value is unknown and is somewhere between zero and the detection limit. Adopting this convention facilitates statistical analyses, and the use of low detection limits potential problems with the convention. However, text discussions of individual contaminants document detection limits and denote frequencies of instances with which the contaminant was below detection limits.

Correlations between variables were checked with Spearman rank correlation coefficients for primarily straight line correlations and plotting for other types of associations.

RESULTS AND DISCUSSION

Comparisons of Contaminant Concentrations

It is not a simple task to accurately measure concentrations of contaminants in tissues of fish and wildlife. However, once the concentrations have been correctly measured, it is sometimes even more difficult to determine what these concentrations mean to the well-being of the organism or to predatory species of fish and wildlife which may consume the organism. Detailed information on this subject is sparse.

There are no uniformly accepted standards for tolerable tissue concentrations of contaminants which will protect fish and wildlife and the predators which consume them. Instead, there is a hodgepodge of action and alert levels proposed by various agencies and experts for specific rather than uniform applications.

Some of these action or alert levels are based on fillet (edible to humans) samples, while others are based on whole-body samples. Some relate to fish only. For the contaminants which have been relatively extensively studied, like PCBs, we have many action or alert levels for comparison with our residue data and therefore our text discussions of these contaminants are relatively long. Very few or no alert levels for fish and wildlife residues have been proposed for many other contaminants, especially those contaminants whose effects have not been well publicized or studied. We know less about the potential meaning of these residues and therefore our text discussions of them are relatively short.

Given these facts, we have presented our results in contrast to as many action or alert levels as we could find. Our goal in doing this is to show the ranges of values for different types of samples and to shed as much light as possible on the meaning of our results. Since this initial exploratory survey utilized so many different species and tissue types, we have presented most of our data in the text rather than in tables to highlight the potential significance of elevated levels and to insure that comparisons utilize samples which are similar enough to be comparable.

Various species and tissue types are different in their efficiency at accumulating contaminants. For example, most organic contaminants tend to accumulate in fatty tissues, some metals tend to concentrate to higher levels in clams than in fish, and only a few contaminants have a tendency to concentrate in muscle (fillet) samples. Therefore, when comparing contamination levels at different sites, the reader is cautioned not to directly compare whole-body contaminant concentrations with fillet (edible tissue) concentrations, nor to make comparisons involving two different species. However, when ranking different species and tissue types at a single station to determine which ones are the most efficient at bioaccumulating a given contaminant, all species and tissue types may be compared with each other. To facilitate both types of comparisons and help circumvent invalid comparisons, we have specified site locations, species, and tissue types in all discussions of contaminant concentrations.

Six types of comparative levels for concentrations of contaminants in fish and wildlife tissues are utilized in this report: Food and Drug Administration (FDA) action levels for human food, FDA action levels for animal feed, predator protection levels, predator alert levels, national mean (NCBP) levels, and gradient monitoring levels.

FDA Action Levels for Human Food are the standards utilized by the FDA for protection of human health in edible portions of fish and wildlife consumed by humans. Action levels for human food have been proposed for only a few of the contaminants listed in this report. In fish, action levels for human food relate to contaminant concentrations in fillets or muscle-only tissues rather than fatty tissues, bone tissues, or whole-body samples. Action levels represent limits at or above which FDA will take legal action to remove adulterated products from the market.

Our study was designed to address impacts of contaminants on fish and wildlife rather than human health effects, so we did not utilize fillet samples of sportfish. Elevated contaminant levels in whole-body or fat-only samples of turtles or minnows do not necessarily equate to elevated levels in edible tissues of sportfish. Therefore, no conclusions about human health effects of consuming fish from the Trinity should be drawn solely from our data.

FDA Action Levels for Animal Feed are more directly comparable to many of the values listed in this report than are FDA action levels for human food. Action levels for animal feed are the standards utilized by the FDA for the prevention of bioaccumulation of harmful levels of a contaminant in animals as a result of consuming animal feed. Action levels for animal feed have been proposed for only a few of the contaminants listed in this report. In fish and wildlife, action levels for animal feed relate most closely to contaminant concentrations in whole-body samples.

Predator Protection Levels are the maximum concentrations recommended by various experts (designated in the text) for whole-body tissues of fish and wildlife prey species, to provide some measure of protection for the predatory species of fish and wildlife which are consuming them. Predator protection levels have been proposed for even fewer contaminants than FDA action levels. Unlike FDA action levels for human food, predator protection levels should be compared to whole-body contaminant concentrations.

Predator Alert Levels are levels of potential concern developed in this report based on derivations from human health standards. Predator alert levels are provided for illustrative and discussion purposes rather than for regulatory purposes.

We derived predator alert levels for three of the most hazardous and ubiquitous classes of contaminants found in Trinity River fish and wildlife, PCBs, mercury, and chlordane. The derivations of predator alert levels are meant to demonstrate the difficulty of developing "safe levels" and to demonstrate why lower levels of contaminants may be necessary to protect fish and wildlife predators than to protect humans. Predator alert levels should be compared to whole-body concentrations.

Mean NCBP Levels represent the geometric mean of whole-body concentrations of contaminants in fish, as reported in recent years by the National Contaminant Biomonitoring Program (NCBP) of the U.S. Fish and Wildlife Service. Not all contaminants analyzed in this report are included in the NCBP program. However, for some contaminants there are no action or alert levels and the NCBP mean is our only point of reference for comparison.

Gradient Monitoring Levels were used to measure the degree of contamination at various sites. As such, gradient monitoring levels are relative measures for comparison with each other rather than absolute measures for a comparison with an action or alert level.

Gradient monitoring levels utilized in this report are mosquitofish whole-body concentrations. Mosquitofish are not particularly effective accumulators of organic contaminants and therefore would not be apt to exceed action or alert levels for organics except at very severely polluted sites. However, mosquitofish do accumulate at least low levels of all contaminants measured and were found at all of our sampling sites. Therefore, we used them to measure gradients of contaminant concentrations as we moved downstream (see individual discussions and graphs for most contaminants) or among sites affected by different types of runoff.

ORGANIC CONTAMINANTS

Combined (Total) PCBs

Due to their potency and widespread distribution, PCBs are one of the few contaminants for which we have several action and alert levels with which to compare our results. PCBs are very stable compounds which belong to a group of chemicals known as the arene halides, considered by some to be the most hazardous group of chemicals typically found in fish [5].

Liver damage, carcinogenic effects, birth defects, and reproductive problems have been documented for PCBs in mammals such as rodents and monkeys [10,14,57]. PCBs have been found to be carcinogenic in rats [109,114] and mice [109]. In minks and monkeys, some PCB mixtures have been found to cause spontaneous abortions [114]. PCB mixtures have also been shown to be fetotoxic in rodents, causing resorptions [114]. However, some experts believe that many of the mammalian or human effects which have ascribed to PCBs may actually be caused by chlorinated dibenzofurans [114]. Dibenzofurans are very hazardous chemicals which are closely related to PCBs and often occur as contaminants in PCB mixtures [114].

Six PCB compounds are listed by the Environmental Protection Agency among 65 priority pollutants [58]. PCBs are also listed among the 25 hazardous substances thought to pose the most significant potential threat to human health at superfund sites on the national priority list [93].

Fish and other aquatic life bioaccumulate PCBs through the gills and through the foodchain [67]. Bioturbation (stirring) of bottom sediments by fish increases desorption of PCBs from sediments and subsequent bioaccumulation from the water [95].

Due to their ability to biomagnify (increase in concentration as they move to higher levels in the food chain), contaminants such as PCBs, mercury, and chlordane are particularly hazardous to predators such as bass, sunfish, river otters, herons, egrets, and eagles. Predatory fish and wildlife are at risk since such a large percentage of their diet consists of other species of fish and wildlife.

The significant presence of biomagnifying contaminants such as PCBs in the Trinity River is of particular concern because of the use of the river south of Dallas by bald eagles, an endangered species. Eagles are long-lived and predatory, feeding primarily on fish, waterfowl, and carrion. Bottom feeding fish common in the Trinity River, including channel catfish, carp, and flathead catfish, make up a significant portion of an eagle's diet [44].

Carrion available during recurrent fish kills increase the risk from PCB contamination, since eagles may be eating larger fish (which typically have higher levels of PCBs) than normal. Birds of prey such as eagles are susceptible to PCB effects on reproductive hormones [15], lowered sperm counts [16], decreased reproductive success [17], less vigorous behavior [17], and hatching failure [18]. Many of the effects documented above for raptors may also be occurring in other types of birds, although specific information is lacking on most species.

In various fish species, exposure to PCBs has been shown to reduce reproductive success [10] or increase susceptibility to disease [95]. Thus, excessive body burdens of PCBs in prey species, particularly in combination with excessive body burdens of other contaminants known to be harmful to predators, may be adversely impacting predatory species of fish in the Trinity.

PCBs have been widely used as lubricants, insulators, and coolants [67]. Other known sources of PCBs include electrical transformers, capacitors, heat transfer fluids, railroad yards, electrical utilities, oils used for dust suppression, grain elevators, packing plants, scrap and salvage operations including oil and metal recyclers, general manufacturing facilities, electrical equipment in office or industrial buildings, and some types of high tech electronics manufacturing facilities [10,60]. Prior to 1971, PCBs were also used in carbonless copy paper, hydraulic fluid, and paints [114]. As a result of current regulatory restrictions, contaminated fish are the primary food source of PCBs for humans today [114].

In storm drains of downtown areas of large cities, a frequently suspected source of PCBs is large electrical transformers. There are large transformers scattered throughout cities, and any large city has had experience with transformers leaking or occasionally breaking (Captain Gerald Richards, Hazardous Materials Response Team, Fort Worth Fire Department, personal communication).

Fires can rupture transformers. The Environmental Protection Agency has concluded that fires in transformers containing PCBs pose risks to humans and the environment and is continuing a gradual reduction of PCB use in transformers [117]. Combustion of PCB contaminated oils can produce dibenzofurans and other hazardous chemicals. Dibenzofurans are already present as contaminants in some PCB mixtures, so fires would provide an additional source. Combustion of (formerly common) transformer mixtures of PCBs and trichlorobenzene also produces dioxins (Gill Addis, Electric Power Research Institute, personal communication).

Some transformers rupture in transportation accidents, and storms often knock down power line transformers and rupture them [115]. Storm water transport of PCB-contaminated soils or oils is one potential mechanism by which PCBs could move from transformer sites into storm drains.

PCBs are a good example of urban runoff contaminants which may be more effectively reduced by simple efforts to find and eliminate sources and contaminated soils at spill sites rather than by spending more money to further reduce contaminant concentrations in effluents of large sewage treatment plants. Storm drain investigative efforts such as the one now being conducted by the Fort Worth Health Department's Storm Drain Team provide a model "first step" effort which might be copied by other cities wishing to reduce PCBs and other urban runoff contaminants.

Municipal landfills are an additional potential source of PCBs in the Trinity River in the Dallas/Fort Worth area [85,Kirk Brown, Texas A&M University, personal communication]. The potential for PCB contamination of the river from this source is increased by the high concentration of electronic manufacturing facilities in the area, the previous lack of controls for dumping industrial items in municipal landfills [85], and the existence of many municipal landfills located within Trinity River floodplains. In addition to the potential for leachate to enter the river from adjacent landfills, underground flow connections in sandy soils and bank erosion provide additional potential pathways for transport of PCBs or PCB-contaminated soils from the landfills to the river. In municipal landfills, capacitors, transformers, older fluorescent light ballasts (those containing capacitors), slick (magazine) paper, old plastic, and industrial equipment are additional potential sources of PCBs (Kirk Brown, Texas A&M University, personal communication).

The city of Fort Worth recently had to remove many drums of PCB-contaminated waste that had been illegally dumped in its landfill (Gene Rattan, City of Fort Worth, personal communication). Past or illegal disposal of industrial wastes is thought to be the primary source of PCBs in municipal landfill leachates [85].

Total PCBs were found above the detection limit (0.05 mg/kg) in 48 of 64 Trinity River fish or wildlife tissue samples.

Maximum Level: The highest concentration of PCBs detected in fish and wildlife tissues in our study was 20 mg/kg in a whole-body composite sample of four spiny softshell turtles from site no. 18, a storm drain canal in downtown Fort Worth. These turtles were collected from the only site on the Trinity where we have observed anyone fishing specifically for turtles. This highly elevated level was 250 times higher than the 0.08 mg/kg found in the same species from site 1, our control/reference site, and was confirmed by duplicate analysis and GC/mass spectrometry. It is also by far the highest level our agency has reported for softshell turtles from three sites in Texas and one in Arizona [65,79, Gerry Jackson, U.S. Fish and Wildlife Service, personal communication].

During our 1985 collections, no sport fish were present at site 18. Since softshell turtles eat fish, their eating habits mimic predatory fish more than most turtles. On the other hand, our softshell turtle samples usually had at least twice as much lipid content than even fatty species of fish. Therefore, higher PCB levels would be expected in softshell turtles.

FDA Action Levels: The FDA action level for PCBs in fish or shellfish to be consumed by humans is 2.0 mg/kg. The Texas Department of Water Resources previously reported PCB concentrations from 3.2 to 4.51 mg/kg from catfish, carp, and longnose gar from our site 9 [3].

In our study, only turtle samples were elevated above 2.0 mg/kg total PCBs. Included were the soft shell turtles from site 18 (see discussion above), one Mississippi map turtle whole-body sample (3.7 mg/kg) from site 7, a composite sample (3.5 mg/kg) of spiny softshell turtles from site 11, and one fatty tissue sample (2.9 mg/kg) from a snapping turtle from site 15.

Since there is no FDA action level for PCBs in turtles and since none of our fish samples were edible portions of sport fish, no direct comparison with the FDA action level can be made with our data. However, it is interesting to note that the Food Safety and Inspection Service of the U.S. Department of Agriculture uses 3 mg/kg as a maximum concentration of total PCBs allowable in fat tissues of animal meat and poultry products bound for human consumption [121].

The human consumption of freshwater turtles in Texas appears to be limited. However, certain groups of people in Texas do enjoy catching and eating turtles and increased consumption is being encouraged by some [13].

Predator Protection Levels: Although the highest concentrations of PCBs are often found in turtles, much lower concentrations of PCBs in fish can cause problems to fish-eating predators. Reproductive toxicity in fish has been shown to occur at whole-body PCB residues as low as 0.4 mg/kg PCBs [10]. The National Academy of Sciences recommended as early as 1973 that for protection of aquatic life, residues of PCBs in body tissues should not exceed 0.5 mg/kg [12].

Trinity River samples having PCB concentrations above 0.5 mg/kg but less than 2.0 mg/kg included: from site 11, 3 samples of carp, one sample of blue catfish, one sample of fatty tissue dissected from red-eared turtles, and one sample of fatty tissue dissected from a Mississippi map turtle; from site 14, one whole-body sample of channel catfish, one whole-body sample of fresh water drum, and one composite whole-body sample of two Mississippi map turtles; from site 6, one whole-body sample of mosquitofish; from site 24, a composite sample of 4 whole-body smallmouth buffalo fish collected by the Texas Parks and Wildlife Department during the first fish kill of the summer of 1985; and from site 18, a composite sample of fatty tissue dissected from 5 red-eared slider turtles.

Based on reproductive effects and more recent information than was available to the National Academy of Sciences, the Great Lakes International Joint Commission recommends 0.1 mg/kg total PCBs as a whole-body fish residue objective not to be exceeded to protect birds and mammals which consume fish [59]. All but 27 of our 64 Trinity River tissue samples exceeded the 0.1 mg/kg total PCBs level, and most of the 27 which were lower than 0.1 mg/kg were either mosquitofish or other lean tissues not considered to be particularly effective at concentrating organic contaminants.

Predator Alert Level: It may surprise some readers that concentrations of contaminants which are lower than FDA action levels for protection of humans have been recommended for the purpose of protection of wildlife. The following example of a simple derivation of an equivalent fish and wildlife predator alert level, developed from a human health standard, may help illustrate why lower standards may be required for protection of fish and wildlife.

Assuming fish typically are eaten by humans at no more than 3 of 21 meals per week, we can calculate that fish are consumed at 3 divided by 21, or 14.28 % of all meals. Further assuming that fish usually account for no more than half of the food at each of those meals, a typical maximum percentage of fish in the human weekly diet could be estimated as 14.28% x 0.5 or 7.14% of the diet.

On the other hand, predators such as bass may be eating other fish and wildlife exclusively, and the tendency of contaminants like PCBs to bioaccumulate in predators or biomagnify in the food chain make a lower standard necessary. Fish and wildlife predators usually consume the entire body of a prey species rather than fillets. Whole-body samples typically have at least twice the concentration of fat (lipids) as do fillets, an important consideration since PCBs tend to accumulate in fatty tissues. It is not surprising, therefore, that whole-body samples of fish usually have 2 to 3 times higher concentrations of PCBs than do fillet (edible portion) samples [67].

Dividing the 7.14% level by 2 to compensate for the difference between whole-body and muscle (fillet) concentrations would yield a fish and wildlife application factor of 0.036. Multiplying 0.036 by the 2.0 FDA action level for human food would yield an alert level for protection of fish and wildlife predators of 0.072 mg/kg, a level exceeded by all but 22 of the 64 Trinity River samples analyzed for PCBs.

Discussing the above-summarized rationale with various experts prompted two opposite responses. Those who felt 0.072 mg/kg might be too low pointed out that individual predator species, whether a bird, fish, or mammal, may be more resistant to effects of PCBs than humans. In most cases no definitive comparison can be made between humans and various species of fish and wildlife, because of lack of sufficient data.

Those who felt 0.072 mg/kg alert level for PCBs was too high noted that there is no recognized safe level for a human carcinogen, that some species of fish and wildlife also get cancer, and that a level of zero PCBs would be required for maximum protection [60]. This line of reasoning holds that it is impossible to define a truly safe level for directive chemicals such as PCBs and that less of these chemicals is always better than more of them [11]. Some otherwise hardy species of fish are relatively susceptible to carcinogens [81,82].

Those reviewers who thought 2.0 mg/kg might be too high a starting point were nevertheless generally amenable to the idea of developing fish and wildlife alert levels from human standards. Human health standards are usually derived to a large degree from experimental data from animals so these reviewers believe we should not ignore their potential applicability to fish and wildlife.

One reviewer (Dick Ruelle, Fish and Wildlife Service) suggested that 0.05 mg/kg be used as the starting point rather than using the 2.0 mg/kg FDA action level for human food. His reasoning was that risk assessment workers in the Environmental Protection Agency and other agencies were starting to use 0.05 mg/kg as a protective level for humans eating fish frequently to protect against cancer and other risks. He also pointed out that the 2.0 mg/kg was designed to protect humans given the assumption that fish from PCB-polluted areas (such as the Great Lakes) were distributed nationwide to spread around the risk. If 0.05 mg/kg were used as the starting point, the alert level for protection of fish and wildlife predators would be 0.05 x 0.036 (the application factor derived above) = 0.0018 mg/kg PCBs. This is a concentration well below our detection limit of 0.05 mg/kg.

However, it is not our purpose to propose either 0.072 or 0.0018 mg/kg of total PCBs as predator alert levels. We include their simplified derivations only as an illustration of 1) why more rigorous alert levels for the protection of fish and wildlife predators may eventually need to be developed, 2) why they might logically be lower than standards which have been recommended for protection of human health, 3) the great diversity of opinion amongst experts on how to develop alert levels, and 4) the general difficulty of determining "safe" levels for directive contaminants such as PCBs [11].

At this time we recommend that the previously-mentioned predator protection level (0.1 mg/kg total PCBs) adopted by the International Joint Commission be utilized as an interim predator alert level for discussion purposes [59]. Some of the key species of fish and wildlife that the International Joint Commission level is designed to protect are present in the Trinity River. In the absence of additional information to the contrary, we see no reason why its use for freshwater systems in Texas is any less appropriate than its use for the Great Lakes.

It is interesting to note, however, that the 0.1 mg/kg fish goal level developed by the International Joint Commission is close to the 0.072 mg/kg level developed in our illustration. The derivation of the International Joint Commission goal level was based on reproductive effects on fish and wildlife, whereas the 2.0 mg/kg FDA action level used to derive our theoretical human health equivalent level was developed with the stated goal of striking a balance between adequate protection of human health versus causing excessive loss of food for American consumers [14].

Among small mammals occurring along the Trinity, mink are one of the few species for which we have appreciable data. Mink are known to be highly susceptible to PCB effects. Diets with PCB concentrations as low as 0.1 mg/kg caused death and reproductive toxicity in mink [10]. This provides additional support for not using a level above 0.1 mg/kg as a interim recommendation for protection of fish and wildlife predators. As mentioned in the previous section, concentrations of total PCBs in all but 27 of our 64 Trinity River tissue samples exceeded 0.1 mg/kg.

Gradient Monitoring Levels: In the mainstem of the Trinity River, mosquitofish body burdens of PCBs rose steadily from just upstream of the city center of Fort Worth (river mile 529.2) to the highest value (0.71 mg/kg) in far eastern Fort Worth, just upstream of the first large sewage treatment plant (Figure 3). The highest concentration of PCBs in any species was also found in downtown Fort Worth (see discussion above).

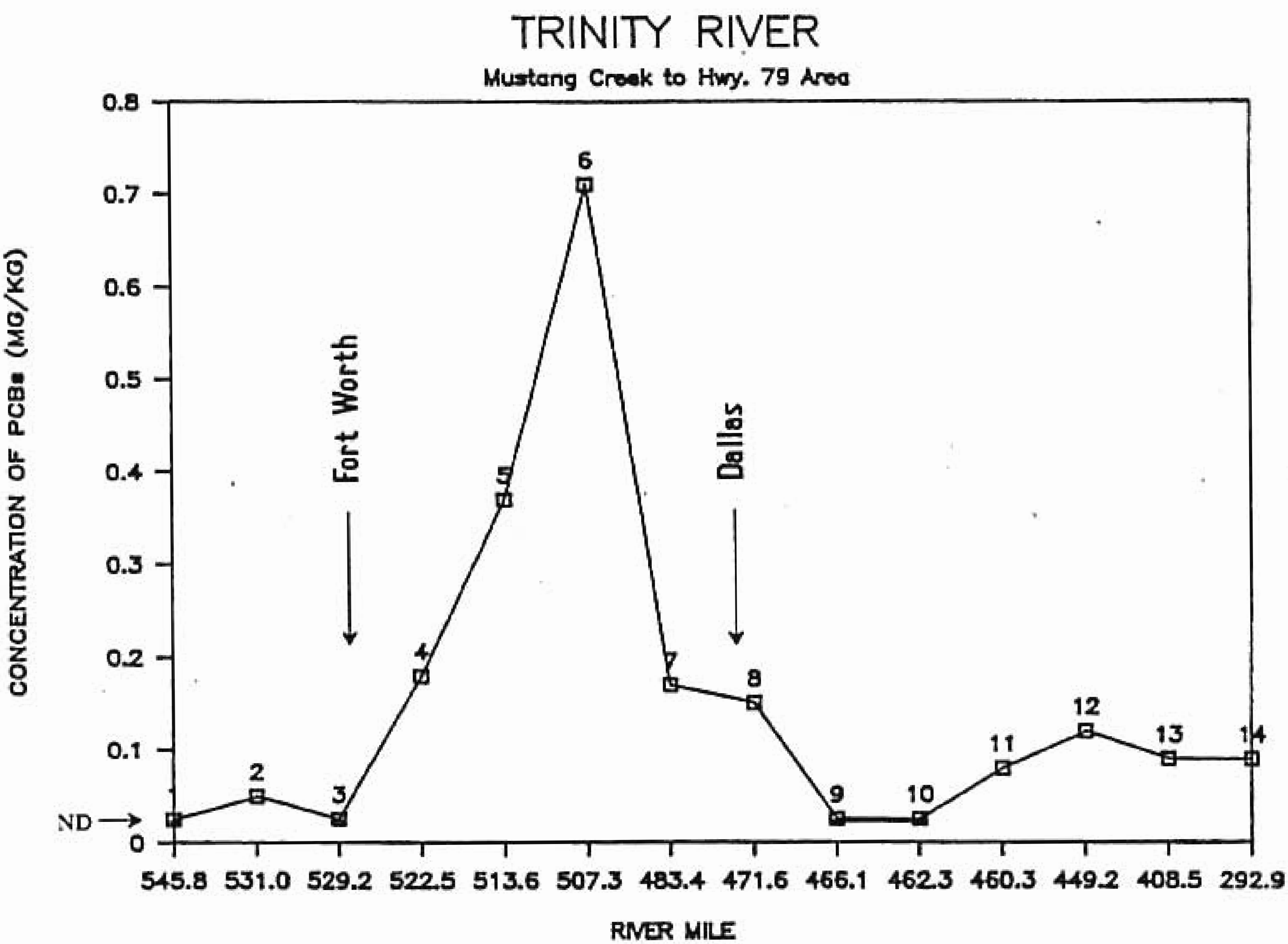


Fig. 3. Wet-weight concentrations of PCBs in whole-body samples of mosquitofish by river mile (site numbers are printed above each point, ND=statistical convention recorded for those values below the detection limit.

Previous studies have documented that concentrations of PCBs in riverine fish and wildlife were highest closest to the source of PCBs [67]. PCB residues in carp were generally proportional to sediment levels in a section of the Mississippi River in Minnesota [95].

In a 1977 Texas Water Quality Board study, elevated concentrations of PCBs were found in sediments from downtown Fort Worth and downtown Dallas [74]. This is similar to the situation found on the Mississippi River, where elevated concentrations of PCBs are found downstream of each major metropolitan area (Dick Ruelle, U.S. Fish and Wildlife Service, personal communication).

An analysis of mosquitofish from all sites (creeks, storm drains, and the mainstem of the river) revealed that areas strongly affected by industrial runoff had significantly higher concentrations of PCBs than did sites receiving little or no runoff from industrial areas.

An analysis of all mosquitofish data showed that sites having substantial runoff from downtown Fort Worth or downtown Dallas had statistically significant higher levels of PCBs than sites having little or no runoff from the downtown areas. This trend was confirmed in a second analysis, in which all sites were grouped according to location type. Those sites classified as rural or suburban and above the influence of either downtown or industrial areas had significantly lower levels of PCBs in mosquitofish than other sites.

Although PCBs are common urban contaminants, PCB levels in fish and wildlife have been dropping nationwide for the last 15 years. PCBs are sometimes absent from fish and wildlife samples from rural areas. For example, no PCBs were detected in 27 samples of fish, turtles, and sediment from the rural Rio Grande River at Big Bend National Park [65].

No significant statistical correlation existed between PCB levels and either the presence of raw sewage or chlorinated, treated sewage. The Trinity River below river mile 507.3 (see figure 3) was highly diluted by chlorinated, treated sewage at the time of our collections and PCBs are usually not discharged in high amounts by area sewage treatment plants. Therefore, the dilution factor may be depressing concentrations of PCBs in the Dallas area and preventing a peak similar to the one we observed in Fort Worth.

Nevertheless, PCB levels in Trinity River mosquitofish were high enough to warrant concern for fish and wildlife predators as far south as Highway 79, and much higher values were reported south of Dallas for other species. Therefore, dilution from sewage effluents evidently did not prevent bioaccumulation of PCBs in fish and wildlife. Over a period of time, persistent contaminants such as PCBs, which tend to adhere to bottom sediments, slowly move down the river. As repeated rainfall events resuspend and redeposit sediments farther downstream, such contaminants migrate downstream. Algae may also be playing a role in moving PCBs downstream [95].

At the time of the July 26, 1985, fish kill, PCB concentrations measured in water at all three Trinity River fish kill stations were at least 180 times higher than the Environmental Protection Agency's recommended water quality criteria level for protection of aquatic life [56]. The tendency of many PCB effects on fish and wildlife to be of a chronic rather an acute nature argues against PCBs as the primary suspect in the fish kill. However, we cannot rule out the possibility that cumulative stress from PCBs and other toxics may be playing a supporting role in initiating fish kills or in preventing the reestablishment of sport fish in polluted stretches of the river.

Combined Chlordane

The insecticide chlordane is listed by the Environmental Protection Agency as one of 65 priority pollutants [58], is rapidly accumulated by aquatic organisms [76], has a tendency to biomagnify in the food chain, and has been shown to be an animal carcinogen in laboratory tests [76]. In the past chlordane had numerous use patterns as an insecticide. In the last 10 years the largest remaining legal use for chlordane has been as a termiticide.

The terms "chlordane" or "total chlordane" have been used by various authors and agencies to refer to many different things, including *cis*-chlordane; *cis* and *trans*-chlordane; *cis*-chlordane and *trans*-nonachlor; and a combination of *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, oxychlordane, heptachlor epoxide, photoheptachlor, and photo-*cis*-chlordane [9]. It is important to utilize as many chlordane components as possible when trying to gauge the total risk to fish and wildlife, since many of the metabolites are more toxic than some of the parent compounds. For example, oxychlordane is 20 times more toxic than *cis* or *trans*-chlordane to rats. Photoheptachlor, heptachlor epoxide oxychlordane, and photo-*cis*-chlordane are all more toxic to bluegills than are the more commonly reported *cis* or *trans*-chlordane [9].

The best "total chlordane" measure available with our data is the sum of *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, oxychlordane, and heptachlor epoxide, which we will refer to as "combined chlordane" to avoid confusion with the other terms.

Maximum Levels: The highest concentrations of combined chlordane were 2.62 mg/kg in a whole-body composite sample of four spiny softshell turtles from site 18 and 1.07 mg/kg in a whole-body composite sample of 16 spiny softshell turtles from site 11 below Dallas.

FDA Action Level for Human Food: Combined chlordane isomers exceeded 0.3 mg/kg in 7 of our Trinity fish samples. However, our samples were whole-body samples rather than fillet or edible-portion only samples. Therefore, our samples are not comparable to the 0.3 mg/kg FDA action level for fish as human food. However, we do consider whole-body combined chlordane concentrations above 0.3 mg/kg to be notably elevated in comparison with whole-body alert levels (see discussions below). The Food Safety and Inspection Service of the U.S. Department of Agriculture uses 0.3 mg/kg as a maximum chlordane concentration allowable in fat tissues of animal meat and poultry products bound for human consumption [121].

Samples in our study with concentrations of combined chlordane above 0.3 mg/kg included: from site 24 (Highway 287), one composite sample of whole-body smallmouth buffalo fish collected by the Texas Parks and Wildlife Department during the first fish kill of the summer of 1985; from site 14, one composite whole-body sample of 10 freshwater drum and one composite whole-body sample of 5 channel catfish; from site 11, one whole-body composite sample of bullhead minnows, one whole-body sample of longnose gar, three composite samples (5 carp each) of whole-body carp, and one composite whole-body sample of 3 smallmouth buffalo fish.

These elevated chlordane levels confirm previous reports of the Trinity River Authority, which collected a few fish downstream of Dallas and found chlordane elevated above the 0.3 mg/kg level in 7 of 38 samples [42]. The Environmental Protection Agency made spot checks of fish samples collected in 1985 from the Trinity River near the Cedar Creek discharge canal and near the Palestine area. Samples of whole fish from both sites had chlordane levels above 0.3 mg/kg (Mike Bastian, personal communication).

In response to our initial findings and those of other agencies, the Texas Parks and Wildlife Department collected edible (fillet) tissues of fish in August 1987 from 15 sites extending from Beltline Road in Grand Prairie to Highway 21. *Cis*- and *trans*-chlordane were elevated above 0.3 mg/kg in three of those samples, including smallmouth buffalo fish from Beltline Road, longear sunfish from our site 15, and smallmouth buffalo fish from our site 9 (Dave Sager, personal communication). Unlike our samples, the Texas Parks and Wildlife samples were fillet portions of sport fish and were therefore comparable to human food action levels.

FDA Action Level for Animal Feed: In this study, combined chlordane levels exceeded the 0.1 mg/kg FDA action level for animal feed in 30 of 64 samples.

Predator Alert Level: Like total PCBs, combined chlordane is a hazardous mixture of chemicals which tend to accumulate in fatty tissues. A predator alert level for chlordane was derived for illustrative and discussion purposes rather than for regulatory purposes.

We used the 0.036 application factor (see predator alert level discussion in PCB section) to provide a safety margin roughly equivalent to those used for human health considerations. This results in a predator alert level of 0.01 mg/kg (0.036 x 0.3 mg/kg FDA action level for human food) combined chlordane.

Only 5 of 64 Trinity River samples had concentrations below 0.01 mg/kg combined chlordane, even if we counted the "nondetected" observations as zero.

Gradient Monitoring Levels: Concentrations of combined chlordane and individual chlordane components were strongly associated with residential runoff (see sections on residential runoff and each chlordane component). In a rural area, we detected no chlordane components in 27 samples of fish, turtles, and sediment from the Rio Grande River at Big Bend National Park [65]. Our Trinity River data show that at least one chlordane component was found above detection limits in all but 5 of 64 samples. The five samples included three samples from the (rural) control/reference site: a composite sample of four spiny softshell turtles, a composite sample of bullhead minnows, and a composite sample of mosquitofish. The two other samples were from organisms which are not very effective accumulators of organic contaminants, Asian clams (site 26) and Texas cooter turtles (site 18).

cis (alpha)-Chlordane)

Cis-chlordane comprises about 19% of technical chlordane (see discussion on combined chlordane). Chlordane is listed by the Environmental Protection Agency as one of 65 priority pollutants [58].

Cis-chlordane was found above the detection limit (0.01 mg/kg) in 43 of 64 fish and wildlife tissue samples.

Maximum Levels: *Cis*-chlordane was most elevated in one composite whole-body sample (0.63 mg/kg) of four spiny softshell turtles in a storm drain canal (site 18) in downtown Fort Worth. Another high concentration (0.38 mg/kg) was found in one composite sample of bullhead minnows from site 11 below Dallas.

FDA Action Levels for Animal Feed: *Cis*-chlordane was above FDA's animal feed standard of 0.1 mg/kg in 10 samples, including: carp and smallmouth buffalo fish collected at site 24 (Highway 287) during the first fish kill of 1985 and at site 11; fresh water drum from site 14 collected during the second fish kill of 1985; and longnose gar and spiny softshell turtles from site 11.

Gradient Monitoring Levels: *Cis*-chlordane showed a general tendency to increase from upstream to downstream in mosquitofish, with most significant detections downstream of urban areas (Fig. 4). No *cis*-chlordane was detected in rural areas in 1) 27 samples of fish, turtles, and sediment from the Rio Grande River at Big Bend National Park [65], or 2) 90 samples of fish and softshell turtles from the lower Gila River in southwestern Arizona [79].

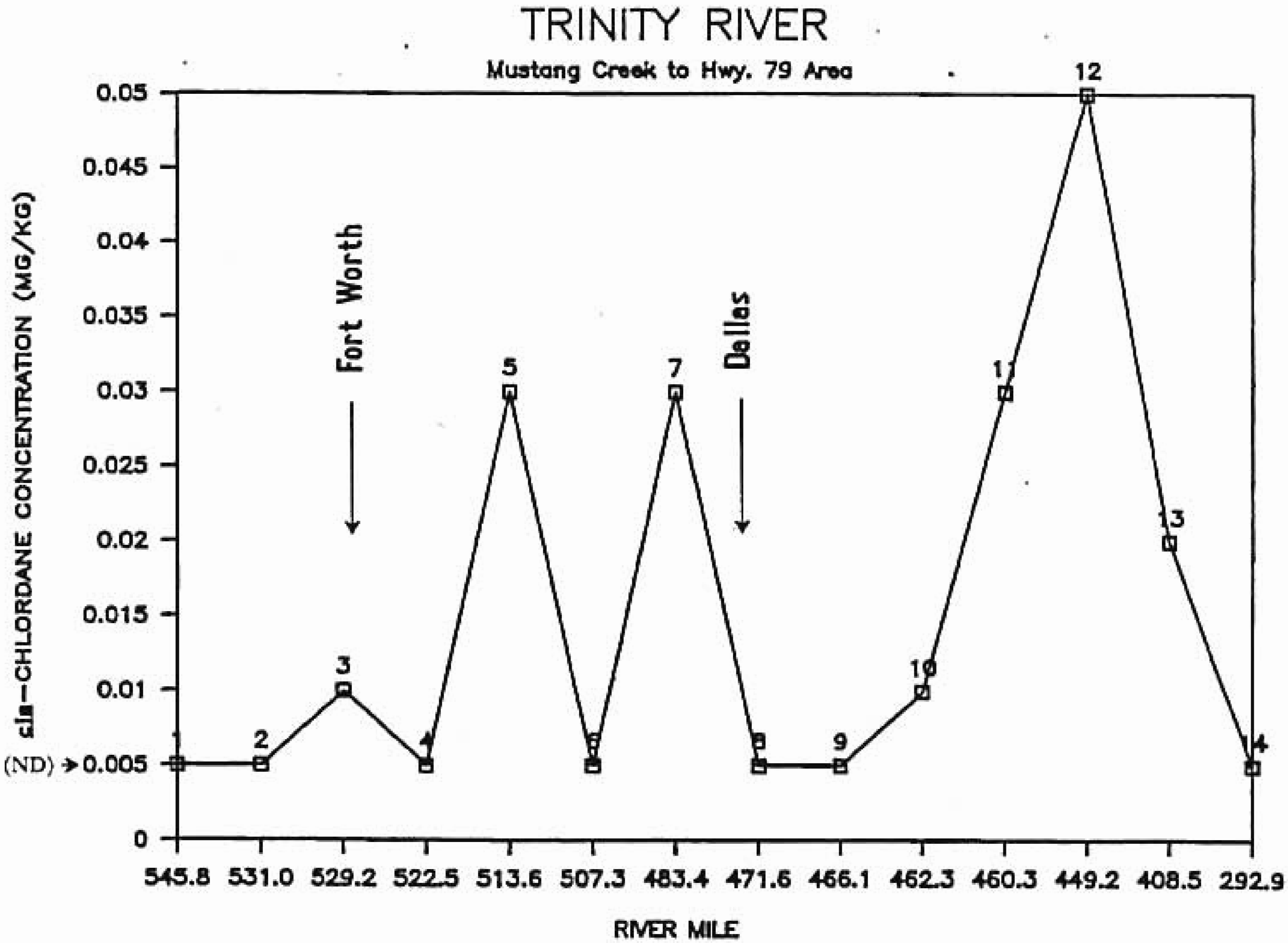


Fig. 4. Wet-weight concentrations of *cis*-chlordane in whole-body samples of mosquitofish by river mile (site numbers are printed above each point, ND=statistical convention recorded for those values below the detection limit).

trans (gamma)-Chlordane

Trans-chlordane comprises about 24% of technical chlordane (see discussion on combined chlordane). Chlordane is listed by the Environmental Protection Agency as one of 65 priority pollutants [58].

Trans-chlordane was found above the detection limit (0.01 mg/kg) in 42 of 64 fish and wildlife tissue samples.

As are the other chlordane isomers, *trans*-chlordane is primarily an urban contaminant. In rural areas, *trans*-chlordane is often not detected in tissues of fish and wildlife. For example, no *trans*-chlordane was detected in 1) 27 samples of fish, turtles, and sediment from the Rio Grande River at Big Bend National Park [65], 2) 90 samples of fish and softshell turtles from the lower Gila River in southwestern Arizona [79], or 3) 16 samples of fish from the San Juan River basin in northwestern New Mexico [86].

Maximum Level: The sample with the highest elevation of *Trans*-chlordane was a composite sample (0.39 mg/kg) of bullhead minnows from site 11 below Dallas.

FDA Action Levels for Animal Feed: *Trans*-chlordane was at or above FDA's animal feed standard of 0.1 mg/kg in 10 additional samples, including: carp and smallmouth buffalo fish collected from site 24 (Highway 287) during the first fish kill of 1985 and at site 11, longnose gar and spiny softshell turtles from site 11, and in one composite sample of four spiny softshell turtles from site 18. Except for the latter sample, all of these locations are downstream of Dallas and Fort Worth.

Trans-chlordane is not as easily eliminated by rats as *cis*-chlordane [57], a factor which may have importance for predatory mammals.

trans-Nonachlor

Nonachlor isomers make up about 7% of technical chlordane (see discussion on combined chlordane). Chlordane is listed by the Environmental Protection Agency as one of 65 priority pollutants [58]. *Trans*-nonachlor is one of the few compounds found in fish which is supertoxic to aquatic invertebrates [5].

Trans-nonachlor was found above the detection limit (0.01 mg/kg) in 22 of 64 fish and wildlife tissue samples.

Maximum Levels: The samples with the highest elevations of *Trans*-nonachlor were composite samples of spiny softshell turtles from site 11 below Dallas (0.37 mg/kg) and site 18 in downtown Fort Worth (1.1 mg/kg).

FDA Action Level for Animal Feed: *Trans* -nonachlor was at or above FDA's animal feed standard of 0.1 mg/kg for chlordane in 7 additional samples, including: one composite fatty tissue sample from three Mississippi map turtles from site 11; one whole-body composite sample of four spiny softshell turtles and one composite sample of fatty tissues dissected from five red-eared slider turtles from site 18; composite samples of five channel catfish, two Mississippi map turtles, and ten freshwater drum from site 14; one whole-body Mississippi map turtle sample from site 7; one composite sample of fatty tissue dissected from five red-eared slider turtles from site 18; and a fatty tissue sample dissected from a common snapping turtle from site 15.

Gradient Monitoring Levels: *Trans*-nonachlor concentrationsin mosquitofish showed a tendency to be elevated primarily at sites in Dallas/Fort Worth, with generally lower values upstream of Fort Worth and downstream of I-20/635 south of Dallas (Fig. 5). In rural areas, *trans*-nonachlor is sometimes not detected in tissues of fish and wildlife. For example, no *trans* -nonachlor was detected in 27 samples of fish, turtles, and sediment from the rural Rio Grande River at Big Bend National Park [65].

cis-Nonachlor

Nonachlor isomers make up about 7% of technical chlordane (see discussion on combined chlordane). Chlordane is listed by the Environmental Protection Agency as one of 65 priority pollutants [58].

Cis-nonachlor was found above the detection limit (0.01mg/kg) in 22 of 64 fish and wildlife tissue samples.

FDA Action Level for Human Food: There is no chlordane action level for turtles. *Cis*-nonachlor concentrations in one composite sample of spiny softshell turtles from site 11 below Dallas was 0.45 mg/kg. The FDA action level for chlordane in fish is 0.3 mg/kg.

FDA Action Level for Animal Feed: *Cis*-nonachlor was at or above FDA's animal feed standard of 0.1 mg/kg for chlordane in 2 additional samples, including one whole-body composite sample of four spiny softshell turtles from site 18 and one composite sample of ten freshwater drum from site 14.

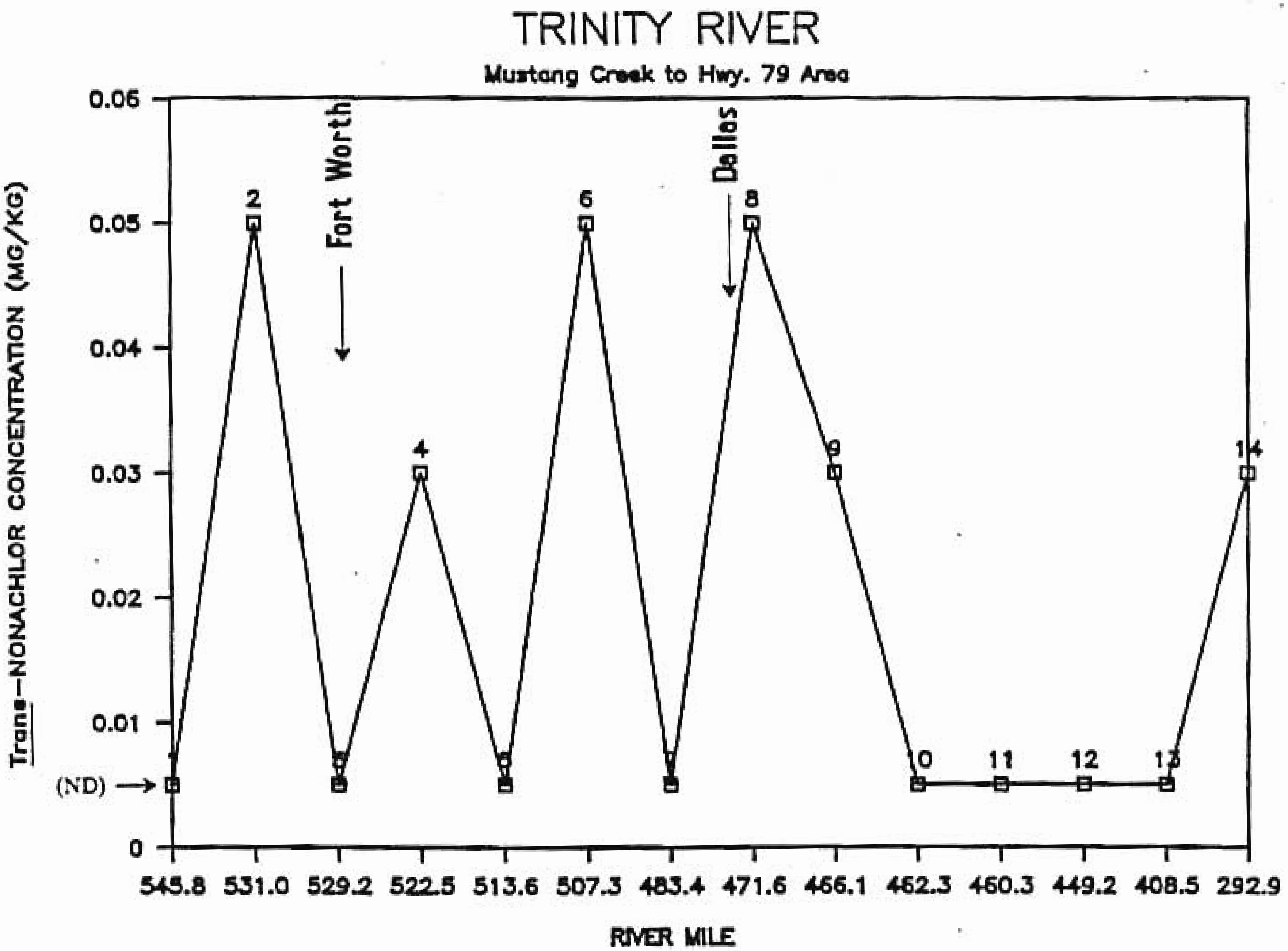


Fig. 5. Wet-weight concentrations of *trans*-nonachlor in whole-body samples of mosquitofish by river mile (site numbers are printed above each point, ND=statistical convention recorded for those values below the detection limit).

<u>Gradient Monitoring Levels</u>: Levels of *cis*-nonachlor in mosquitofish from the mainstem of the Trinity were generally low, with most occurrences downstream of downtown Dallas (Fig. 6). In rural areas, *cis*-nonachlor is often not detected in tissues of fish and wildlife. For example, no *cis*-nonachlor was detected in 1) 27 samples of fish, turtles, and sediment from the Rio Grande River at Big Bend National Park [65], 2) 90 samples of fish and softshell turtles from the lower Gila River in southwestern Arizona [79], or 3) 16 samples of fish from the San Juan River basin in northwestern New Mexico [86].

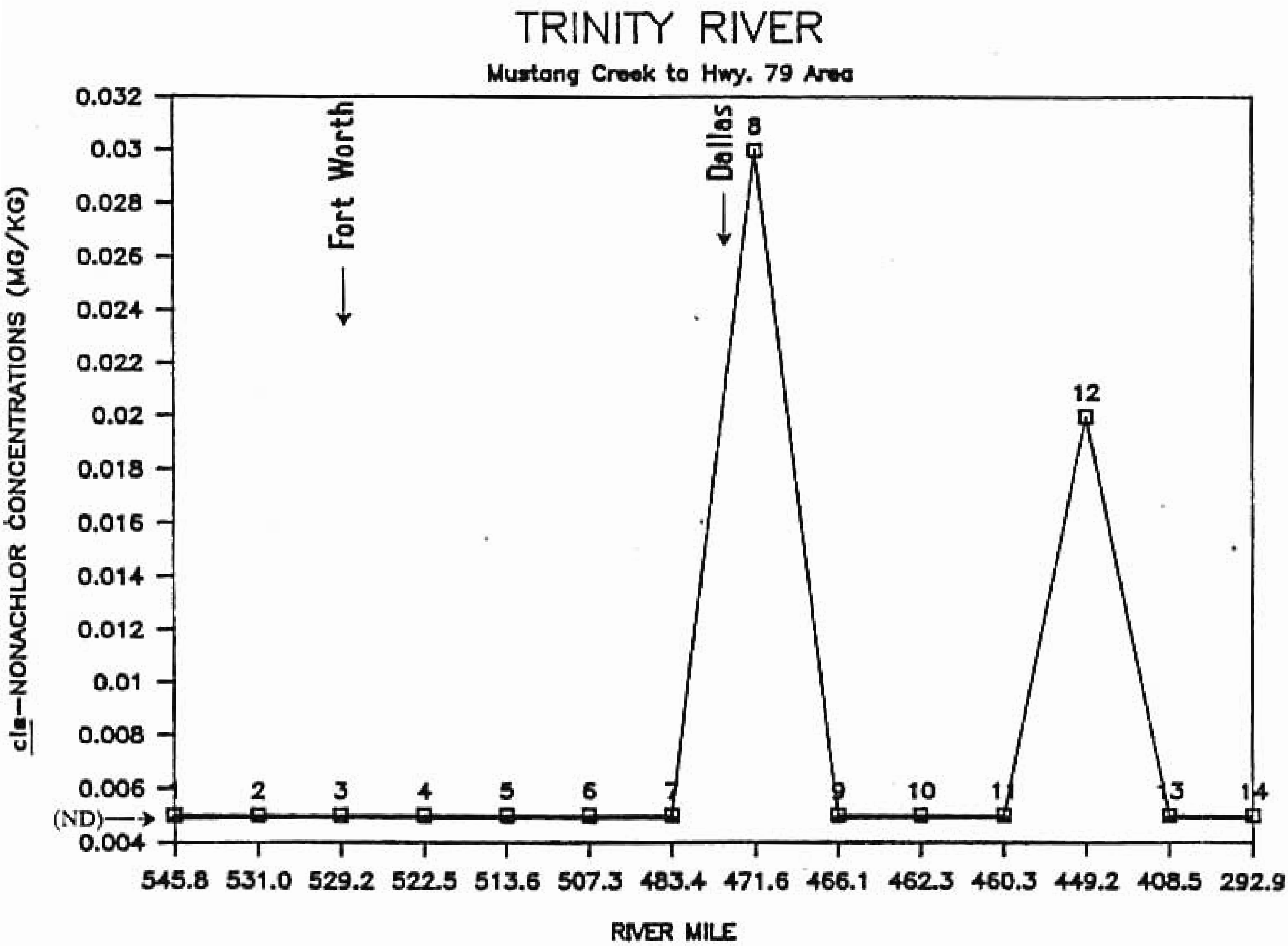


Fig. 6. Wet-weight concentrations of *cis*-nonachlor in whole-body samples of mosquitofish by river mile (site numbers are printed above each point, ND=statistical convention recorded for those values below the detection limit).

Oxychlordane

Oxychlordane is a major breakdown product of technical chlordane. Chlordane is listed by the Environmental Protection Agency as one of 65 priority pollutants [58]. Oxychlordane is important since it is 20 times more toxic than the parent compound and is the most persistent chlordane-related compound in mammalian tissue [57].

Oxychlordane was found above the detection limit (0.01 mg/kg) in 21 of 64 fish and wildlife tissue samples.

FDA Action Level for Animal Feed: Oxychlordane was at or above FDA's animal feed standard of 0.1 mg/kg in three samples, including: one composite sample of four spiny softshell turtles from site 18, one composite sample of fatty tissue from three Mississippi map turtles from site 11, and a fatty tissue sample from a common snapping turtle from site 15.

Gradient Monitoring Levels: Mosquitofish body burdens of oxychlordane were highest from downtown Fort Worth through the mid-cities area to downtown Dallas (Fig. 7). Those sites classified as rural or suburban and above the influence of either downtown or industrial areas had significantly lower levels of oxychlordane in mosquitofish than other sites. In rural areas, oxychlordane is sometimes not detected in tissues of fish and wildlife. For example, no oxychlordane was detected in 27 samples of fish, turtles, and sediment from the rural Rio Grande River at Big Bend National Park [65].

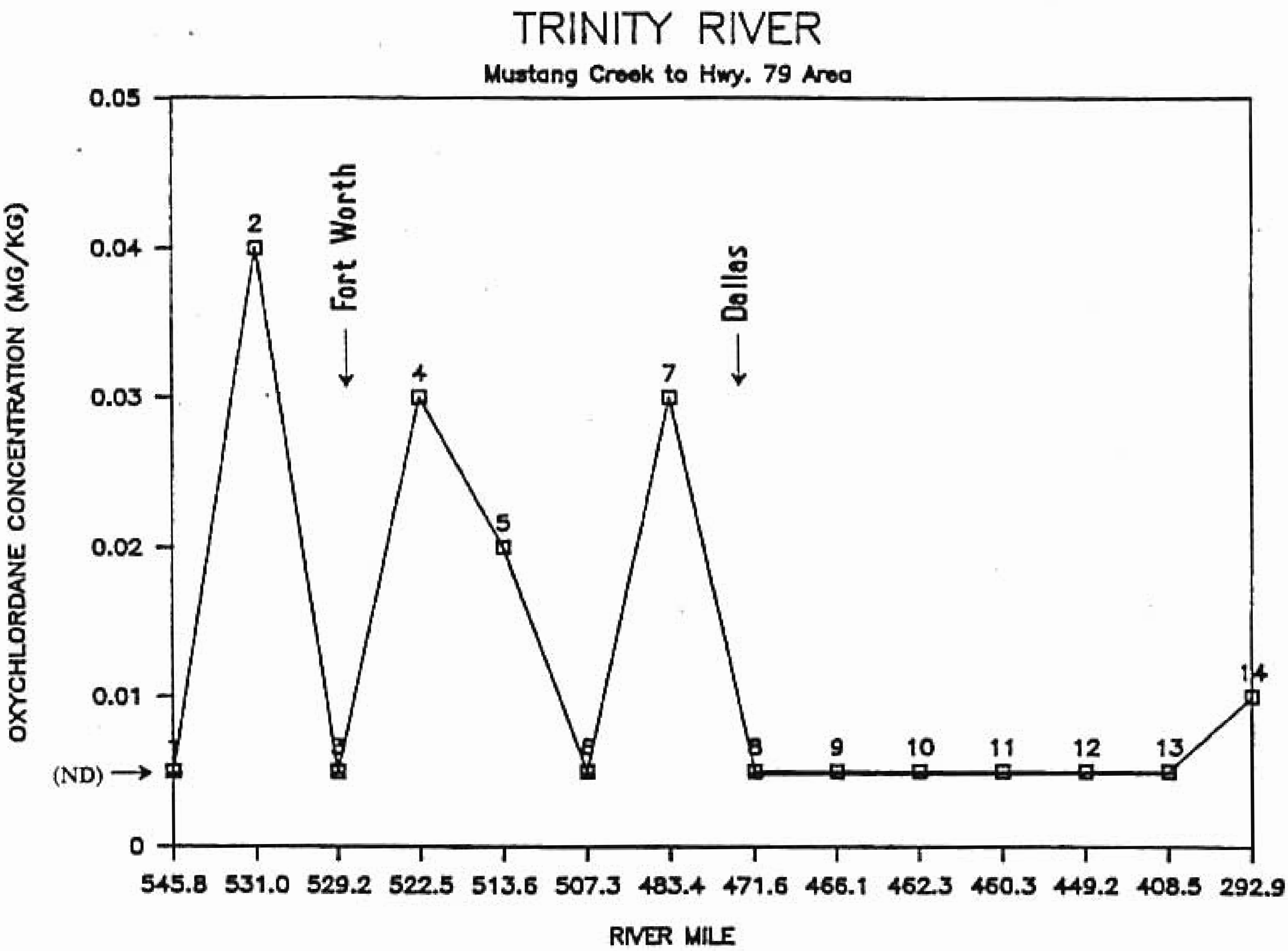


Fig. 7. Wet-weight concentrations of oxychlordane in whole-body samples of mosquitofish by river mile (site numbers are printed above each point, ND=statistical convention recorded for those values below the detection limit).

Heptachlor Epoxide

The insecticide heptachlor is rapidly metabolized into heptachlor epoxide by many organisms. Both heptachlor and heptachlor epoxide are listed by the Environmental Protection Agency among 65 priority pollutants [58]. Heptachlor was formerly widely used as a soil insecticide, crop pesticide, fire ant killer, and termiticide. Heptachlor has a very high carcinogenic potency [85].

Heptachlor is also a minor (<10%), but relatively toxic, component of technical chlordane. Although some authors concluded that most environmental residues of heptachlor epoxide originated in the use of heptachlor, others have concluded that lethal residues in birds originated from technical chlordane [9]. Heptachlor epoxide was found above the detection limit (0.01 mg/kg) in only 5 of 64 tissue samples. The highest level of heptachlor epoxide detected was 0.15 mg/kg in fatty tissues dissected from a composite sample of red-eared turtles from site 1. There is no FDA level for the fat of meat. All five occurrences in our study were below 0.2 mg/kg and all occurred in samples of turtle fat.

This was the only organic contaminant which had a higher concentration in any species at our control/reference site (number 1) than at other sites. The control/reference site was located in a rural area, with pasture the only notable land use. As recently as 1986, there was great new media concern in Texas and several other states about widespread contamination of cow's milk from heptachlor-treated feed grain. Another potential source of heptachlor is its former widespread use in rural areas to control fire ants.

The fact that other chlordane components were not observed at elevated levels suggests that the main source of heptachlor epoxide at this site was not from chlordane. No such suggestion can be made from the other (primarily more urban) sites, many of which also had elevated levels of other chlordane components.

Dieldrin

Since 1974, use of the insecticide dieldrin (and aldrin, which breaks down into dieldrin) has been restricted to termite control and non-food plant treatment [57]. Dieldrin is listed by the Environmental Protection Agency as one of 65 priority pollutants [58]. Dieldrin is also listed among the 25 hazardous substances thought to pose the most significant potential threat to human health at priority superfund sites[93].

Dieldrin produces liver tumors in mice, is toxic to fish, is very persistent, and bioaccumulates in fish [57]. Body burdens of dieldrin decrease a fish's ability to tolerate ammonia, a common toxic compound at Trinity River sites downstream of large sewage treatment plants [57].

Dieldrin was found above the detection limit (0.01 mg/kg) in 49 of 64 fish and wildlife tissue samples. For comparison, no dieldrin was detected in 27 samples of fish, turtles, and sediment from the Rio Grande River at Big Bend National Park [65].

Maximum Levels: Two of our samples had very high levels (at or above 0.3 mg/kg) of dieldrin. Both of these samples were comprised of turtle fat (Mississippi map turtles from site 11, 0.3 mg/kg, and spiny softshell turtles from site 18, 0.35 mg/kg) rather than fish tissue. Apparently there are no action levels specifically for dieldrin in turtles, but the Food Safety and Inspection Service of the U.S. Department of Agriculture uses 0.3 mg/kg as a maximum dieldrin concentration allowable in fat tissues of animal meat and poultry products bound for human consumption [121].

Predator Protection Level: Seven of our whole-body samples were at or above the 0.1 mg/kg maximum dieldrin level recommended by the National Academy of Sciences for the protection of predators [12]. Samples equaling or exceeding this concentration included: from site 24 (Highway 287), a composite sample (0.1 mg/kg) of 4 whole-body smallmouth buffalo fish picked up by the Texas Parks and Wildlife Department during the first fish kill of the summer of 1985; from site 14, one composite whole-body sample (0.1 mg/kg) of 10 freshwater drum picked up during the first fish kill of 1985; from site 11, one composite sample (0.19 mg/kg) of bullhead minnows, one whole-body sample (0.12 mg/kg) of longnose gar, one composite whole-body sample (0.21 mg/kg) of 3 smallmouth buffalo fish, and one composite whole-body sample (0.24 mg/kg) of 16 spiny softshell turtles; and from site 18, the highest dieldrin level of the study (0.35), a composite whole-body sample of 4 spiny softshell turtles.

A few fat-only samples also had concentrations exceeding 0.1 mg/kg dieldrin but were not directly comparable to the National Academy of Science predator protection level since they were not whole-body samples. These included a composite sample (0.30 mg/kg) of fatty tissue dissected from 3 Mississippi Map turtles from site 11 and a composite sample (0.19 mg/kg) of fatty tissue dissected from 5 red-eared turtles from site 18.

Gradient Monitoring Levels: In mosquitofish, dieldrin showed a tendency to increase from upstream of Fort Worth to the Malloy Bridge area downstream of Dallas (Fig. 8). An upstream group of mosquitofish samples from sites 1, 16, and 27 had significantly lower concentrations of dieldrin than a group of mosquitofish samples from all other sites.

In a 1977 Texas Water Quality Board study, elevated concentrations of dieldrin were found in sediments from downtown Fort Worth and downtown Dallas [74]. The highest concentrations of dieldrin (17 to 44 mg/kg) in sediments were from sites 9 to 12 [74]. In both our 1985 study of fish and wildlife and the 1977 Texas Water Quality Board study of sediments, dieldrin was detected at most Dallas/Fort Worth sites and was one of the most frequently encountered organochlorine pesticides at all Trinity River sites [74].

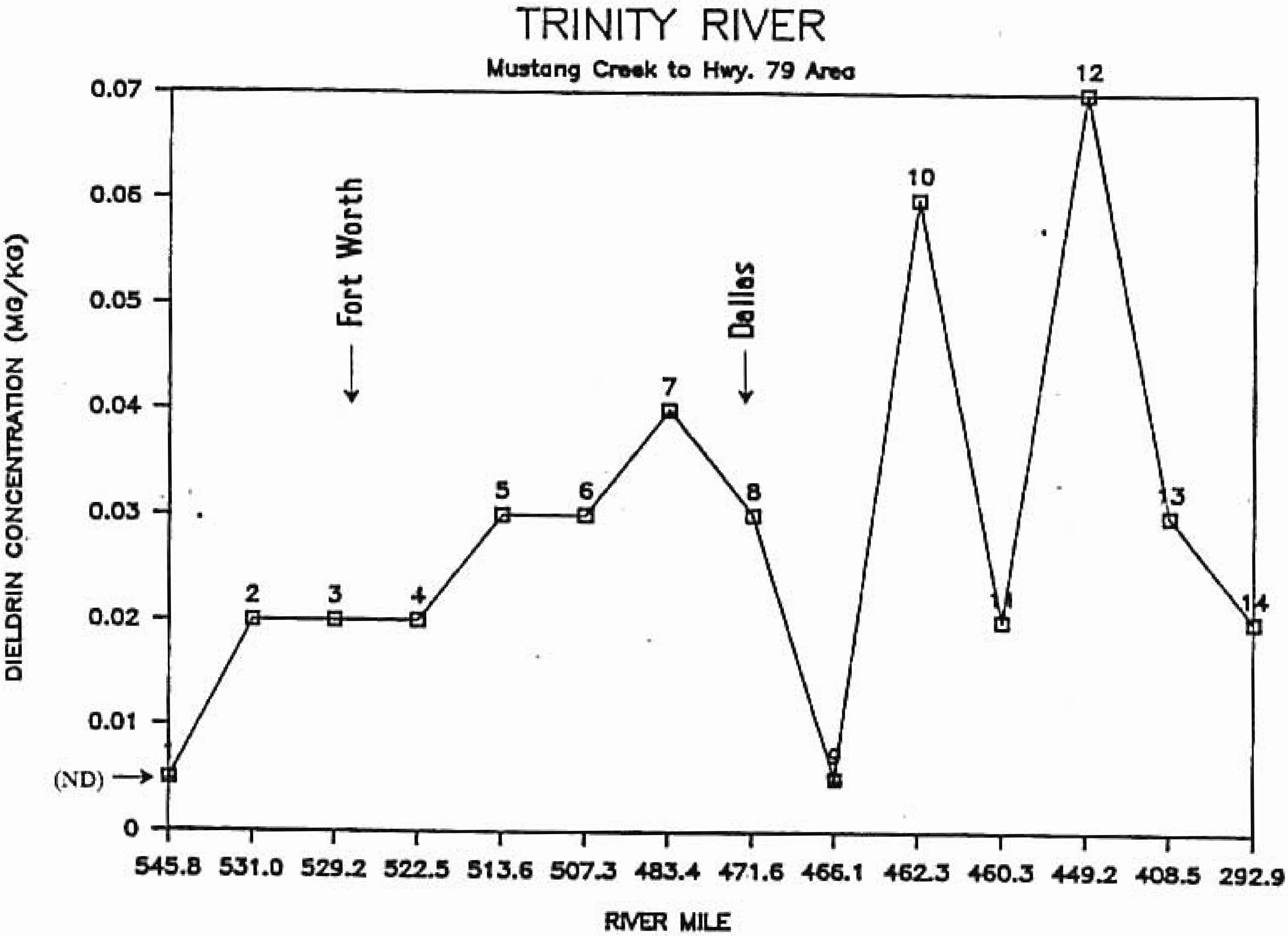


Fig. 8. Wet-weight concentrations of dieldrin in whole-body samples of mosquitofish by river mile (site numbers are printed above each point, ND=statistical convention recorded for those values below the detection limit).

Lindane (*gamma*-BHC)

Lindane is the most toxic BHC isomer to fish. Lindane has a very high carcinogenic potency and has been known to induce tumors in mice and rats [57,85]. Its manufacture has been banned in the U.S., and it is listed by the Environmental Protection Agency as one of 65 priority pollutants [58]. Lindane has few remaining legal uses in the U.S. other than applications in veterinary medicine.

Lindane was found above the detection limit (0.01 mg/kg, wet-weight) in 7 of 64 tissue samples. This is not a high percentage, but lindane's potency and increasing rarity in most locations make this occurrence of interest. All 7 samples having detectable concentrations of lindane were from sites downstream of large sewage treatment plants. The Environmental Protection Agency and the Texas Water Commission have both reported presence of lindane in spot checks of fish tissue and water from sites below Dallas [45].

Lindane is continually degraded and eliminated from the body [57]. No lindane was detected in 27 samples of fish, turtles, and sediment from the Rio Grande River at Big Bend National Park [65]. Nor was any lindane detected in 16 samples of fish collected recently in a fish and wildlife survey of the San Juan River basin near Farmington, New Mexico [86]. A survey of Pennsylvania fish from 48 sites revealed detectable lindane at only one site [57]. Stored lindane is completely eliminated from the body once the source of contamination is removed [57]. Therefore, its presence in fish is cause for concern since it suggests the possibility of a continued source [57].

Since all occurrences in our study and other Trinity River spot checks we are familiar with have been downstream from large sewage treatment plants, it is possible that small amounts of lindane are being formed by chlorination of organics in sewage, as has been documented by the Environmental Protection Agency in other localities [8]. The summertime practice of treating incoming sewage with chlorine to combat odor problems, such as has been done at Fort Worth's Village Creek Sewage Treatment Plant, could exacerbate the potential of forming lindane in four ways: 1) more chlorination points, 2) chlorination of an additional waste stream which is higher in organics, 3) the general ineffectiveness of activated sludge at removing lindane, and 4) the fact that biodegradation in activated sludge is decreased with increases in chlorination [97].

There is a large dilution factor in the final effluent of a 100 million gallon per day facility like the Village Creek Plant. This factor, combined with the infrequency with which sewage plant effluents are checked for priority pollutants, make it difficult to identify very low levels of contaminants such as lindane. In the case of Village Creek Sewage Treatment Plant, prechlorination of the effluent is now restricted to summer season odor control efforts, whereas priority pollutant effluent checks have been conducted in the winter.

Vigorous oxidation of humic materials, such as might occur when sewage treatment plant disinfectants enter a river, sometimes liberates entrapped or complexed toxic pesticides such as lindane [101]. However, further study would be required to determine all sources of lindane residues in the Trinity River.

Maximum Level: The highest levels of lindane found in our study was 0.06 mg/kg in whole-body samples of longnose gar and smallmouth buffalo fish from site 11. Like PCBs, the highest concentrations of lindane tend to be in fatty tissues. Both of the samples having 0.06 mg/kg lindane had lipid percentages exceeding 14%.

Mean NCBP Levels: The national geometric mean of lindane in whole-body samples of fish in the 1981 NCBP program was <0.01 mg/kg [9]. Because of lindane's rapid disappearance from aquatic organisms, little or none has been detected in recent surveys [57].

beta-BHC

Like lindane, *beta*-BHC is an isomer of hexachlorocyclohexane and it is listed by the Environmental Protection Agency as one of 65 priority pollutants [58].

Beta-BHC was found above the detection limit (0.01 mg/kg) in 1 of 64 tissue samples. One sample of fatty tissue from a red-eared turtle collected at site 11 contained 0.01 mg/kg of beta-BHC. Our discussion of *beta*-BHC is shorter than our discussions for most other contaminants, since 1) it was detected in only one sample, 2) we do not consider 0.01 mg/kg to be an especially elevated level for a fatty tissue sample, and 3) we know of no action or concern levels directly comparable to this sample.

Mirex

The insecticide mirex was found above the detection limit (0.01 mg/kg) in only 3 of 64 tissue samples. One sample of fatty tissue from a common snapping turtle collected at site 15 contained 0.01 mg/kg, as did whole-body samples of spiny softshell turtles from sites 11 and 18. These are trace rather than elevated levels [9] and presumably mirex residues will continue to decline since the compound is no longer widely used.

Total Non-DDT Organochlorine Insecticides

Excluding the DDT isomers, the best total oranganochlorine insecticide measure available with our data is the sum of combined chlordane, dieldrin, and lindane.

Predator Protection Level: The National Academy of Sciences recommended that the total of the residues of these chemicals should not exceed 0.1 mg/kg to protect predators [12]. Total non-DDT organochlorine pesticides exceeded the 0.1 mg/kg level in 34 of 64 Trinity River samples. Most samples exceeding 0.1 mg/kg were from fatty (>5% lipids) species or from lean tissues of a variety of fish species collected downstream of Dallas.

Maximum Levels: The highest levels of total non-DDT organochlorines were 2.97 mg/kg in a whole-body composite sample of four spiny softshell turtles from site 18 and 1.37 mg/kg in a whole-body composite sample of 16 spiny softshell turtles from site 11 below Dallas.

DDE

DDE is a breakdown product of DDT, an insecticide which had widespread use before being banned in the U.S. in 1972. DDE is listed by the Environmental Protection Agency as one of 65 priority pollutants [58]. DDE (p',p' DDE) was found above the detection limit (0.01 mg/kg) in all but 3 of 64 fish and wildlife tissue samples.

Maximum Level: The highest value of DDE was 0.85 mg/kg in a composite sample of four spiny softshell turtles from site 18. Higher levels (1.17 mg/kg) had previously been reported by the Texas Water Commission from longnose gar at our site 9.

Mean NCBP Levels: The only Trinity River fish samples exceeding the 0.20 mg/kg national geometric mean [9] for whole-body concentrations of DDE in fish were smallmouth buffalo fish, longnose gar, and carp from the Trinity River downstream of Dallas.

Gradient Monitoring Levels: A group of mosquitofish samples from sites 9, 10, 11, and 12 just downstream of Dallas had significantly higher concentrations of DDE than a group of mosquitofish samples from sites (1, 16, and 27) upstream of Fort Worth or Dallas. Those sites classified as rural or suburban and above the influence of either downtown or industrial areas had significantly lower levels of DDE in mosquitofish than other sites.

Concentrations of DDE in Trinity River fish and wildlife were generally lower than those from the Rio Grande River at Big Bend National Park, where a continued source from the Rio Conchos River is suspected [65]. For example, whole-body concentrations of DDE in all 4 samples of mosquitofish collected in Big Bend National Park exceeded 0.06 mg/kg, while none of the 24 Trinity River samples of mosquitofish exceeded that level.

DDD

DDD was formerly marketed as the pesticide TDE. Like DDE, p, p' DDD (DDD) is a breakdown product of DDT. DDD is listed by the Environmental Protection Agency as one of 65 priority pollutants [58]. DDD (p',p' DDD) was found above the detection limit (0.01 mg/kg) in 27 of 64 fish and wildlife tissue samples.

Maximum Level: The highest concentration of DDD was 0.17 mg/kg in a composite sample of four spiny softshell turtles from site 18.

Mean NCBP Level: The one other value above the 0.07 mg/kg national geometric mean [9] for fish was recorded for a whole-body sample (0.09 mg/kg) of longnose gar from site 11.

Gradient Monitoring Levels: Those sites classified as rural or suburban and above the influence of either downtown or industrial areas had significantly lower levels of DDD in mosquitofish than other sites.

Combined DDT, DDE, and DDD

No DDT was found above the detection limit (0.01 mg/kg) in 64 fish and wildlife tissue samples. Agricultural use of DDT has been banned in the U.S. since 1972 and most remaining residues have presumably broken down into DDE, DDD, and other chemicals. Totals of DDE and DDD detected in Trinity River tissue samples were not elevated compared to national surveys [9].

Maximum Level: The highest value we found for DDE and DDD combined was 1.02 mg/kg in a whole-body sample of spiny softshell turtles from site 18. This was the only value above the 1.0 mg/kg maximum whole-body DDT concentration recommended by the National Academy of Sciences for protection of predators [12].

Gradient Monitoring Levels: Combined values for DDT compounds in samples of Trinity River mosquitofish were significantly lower than comparable samples from the Rio Grande River at Big Bend National Park [65].

Polycyclic Aromatic Hydrocarbons (PAHs)

Twelve of the 14 polycyclic aromatic hydrocarbons (PAHs) analyzed in this study have been listed by the Environmental Protection Agency among 65 priority pollutants [58]. Five of them are also listed among the 25 hazardous substances thought to pose the most significant potential threat to human health at priority superfund sites [93]. PAHs are sometimes referred to as polynuclear aromatic hydrocarbons or as polycyclic aromatic compounds. PAHs are also grouped in a category of chemicals referred to as aromatic hydrocarbons (AHs) in some publications.

Higher weight PAHs include some of the most carcinogenic chemicals known to man. Many PAHs, and several breakdown products of PAHs have been documented to be tumorigenic, teratogenic, and mutagenic to a variety of fish and wildlife, including fish, birds, amphibians, and mammals [40]. Immunosuppressive effects have also been documented in mammals [41]. Aquatic organisms can bioaccumulate some of these compounds [70]. The otherwise hardy brown bullhead, *Ictalurus nebulosus*, is notably susceptible to PAHs, though evidence of cancer may not appear for 2 years after the initial exposure [81,82].

Although some research seems to indicate that interior portions of above-ground vegetables do not accumulate high concentrations of PAHs, plants do translocate PAHs from roots to other plant parts, such as developing shoots [40]. This factor may have significance for herbivorous species of fish and wildlife. Some plants can evidently catabolize benzo(a)pyrene,a PAH which some authors have referred to as the ultimate carcinogen, but metabolic pathways have not been clearly defined. When PAHs do degrade in plants through metabolism, they often break down into even more toxic, carcinogenic, and mutagenic compounds [40]. The PAH biomagnification potential of vegetation in terrestrial and aquatic food chains needs to be measured for a variety of PAHs in both field and laboratory experiments before we will have a complete understanding of these transformations [40].

Metabolic transformations of PAHs into even more hazardous chemicals could also occur in sediments, soils, and various species of fish and wildlife [40,70]. Metabolic degradation of carcinogenic PAHs proceeds very slowly in subsurface soil or sediment environments. This is because these environments are low in oxygen and sunlight [40].

PAHs usually make up 10 to 30% of crude oil and waste crankcase oil [75], with used motor oil typically having much higher concentrations of PAHs than new motor oil [40]. Like several individual PAHs, waste crankcase oil has been shown to be mutagenic and teratogenic [75]. Naphthalene, benzo(a)pyrene, fluorene, and phenanthrene are common PAH components of used motor oil [75]. Among PAHs, benzo(a)pyrene and the approximately 20 other carcinogenic PAHs have been studied more extensively than most other PAHs.

In rural areas a considerable portion of PAHs in streams comes from highways [43]. Citizens or businesses who illegally pour used motor oil into storm drains are polluting urban rivers with PAHs. Aquatic environments also receive PAHs from sewage treatment plants or atmospheric deposition [40].

The heavier PAHs [such as benzo(a)pyrene] are such potent carcinogens that they have been known to produce tumors in test animals from single exposures to very small quantities [40]. Sediment standards are not available for most individual PAHs, but the interim sediment criteria value adopted by EPA for phenanthrene, a non-carcinogenic PAH, is 6.2 ug/gC, equivalent to about 18.9 parts per million [127]. A scientist working on the interagency task force developing these standards has informed us that interim standards for many other PAHs may end up in the 1-20 parts per million range, and indications are that final criteria values may be even lower (Chris Ingersoll, U.S. Fish and Wildlife Service, personal communication).

PAHs were analyzed in only 33 Trinity River samples due to cost. Although PAHs occur in smoked or barbecued fish and can occur naturally in the environment in small concentrations, raw fish from unpolluted areas usually do not contain detectable concentrations of PAHs [40]. Highly elevated concentrations of PAHs in the environment are usually the result of contamination from petroleum products or various industrial or combustion activities [40].

Our understanding of the effects of most PAHs and their hazardous metabolic breakdown products is very incomplete and changing rapidly as research is completed [40]. The environmental effects of the non-carcinogenic PAHs are poorly understood [40]. Given these factors, the best policy for preventing PAH impacts to fish and wildlife is to reduce or eliminate them wherever possible [40].

Maximum Level: The highest PAH concentrations in our study were from a composite whole-body sample of 100 mosquitofish from site 9 at south Loop 12 just downstream of Dallas Central Sewage Treatment plant. The concentrations of total PAHs in mosquitofish from site 9 was 60.79 mg/kg. This is an extremely elevated level and was confirmed by duplicate GC/mass spectrometry analysis.

The individual PAHs detected in this mosquitofish sample (and their respective concentrations in mg/kg listed in parenthesis) included naphthalene (0.19); fluorene (0.50); phenanthrene (7.2); anthracene (1.1); fluoranthene (10); pyrene (8.7); 1, 2-benzanthracene (5.3); chrysene (4.9); benzo(b)fluoranthene (3.5); benzo(k)fluoranthene (3.2); benzo(e)pyrene (1.2); 1,2,5,6-dibenzanthracene (1.6); benzo(g,h,i) perylene (8.1); and benzo(a)pyrene (5.3). These concentrations, including those of the heavier PAHs like benzo(a)pyrene, are much higher than those implicated in a high incidence of liver cancer in bullhead catfish from a severely polluted river in Ohio [40,87]. The next highest concentrations of total PAHs were 0.75 mg/kg, in a composite whole-body sample of smallmouth buffalo fish from site 11 (one mile downstream), and 0.51 mg/kg from a composite whole-body sample of redfin shiners from site 18, a storm drain site in downtown Fort Worth.

At the time of our collections, there was a slick of used motor oil on the surface of the water at site 18. Soon after we notified them of the oil slick at site 18, the City of Fort Worth Health Department discovered that a new car dealer in downtown Fort Worth was illegally dumping used motor oil into a storm drain which flowed directly to the river at that site.

All other elevated levels of total PAHs (>0.1 mg/kg) were also either from sites downstream of Dallas (3 mosquitofish samples, one carp sample, and one longnose gar sample) or from site 18 (one fatty tissue sample dissected from Texas cooter turtles).

Previous studies by other agencies have reported concentrations of PAHs in sediments from site 9 in the 0.5 to 0.7 mg/kg range. Downstream of a creosote superfund site (Eagle Harbor site in Puget Sound), concentrations of total PAHs above 1.0 part per million dry weight had positive correlations with incidence of liver cancer in fish [124,125, confirmed by Don Malins, Pacific Northwest Research Foundation, personal communication]. A total PAH dry weight concentration of 1.0 part per million might typically correspond to a wet weight concentration of about 0.5 parts per million, a total carcinogenic PAH level of 0.083 to 0.166 parts per million and an individual Benzo(a)pyrene concentration of perhaps 0.0267 to 0.0369 parts per million.

If researchers at the Eagle Harbor site had used minimum detection limits for individual PAHs above 0.002 parts per million, they would not have had the low level resolution to determine the 1.0 part per million total PAH level above which PAH concentrations were positively correlated with liver cancer in fish. This provides a convincing argument for using the lowest possible minimum detection limits at sites where one suspects PAHs as important contaminants.

Other researchers have also documented carcinogenic impacts from low levels of PAHs in sediments. For example, sediments from the Buffalo River, New York with concentrations of total carcinogenic PAHs as low as 1.0 mg/kg induced tumors in brown bullhead catfish [40].

All 13 Trinity River samples from relatively clean waters upstream of Dallas or Fort Worth had total PAH levels below 0.05 mg/kg. Concentrations in this range may be the result of atmospheric fallout from nearby urban sources.

Gradient Monitoring Levels: Mosquitofish residues of PAHs are summarized in Table 2. Sites 9, 10, and 15 had mosquitofish levels of PAHs higher than those reported [65] from a Pecos River site which experiences recurrent oil pollution. However, only site 9 had levels above the surprisingly high levels reported from mosquitofish from the Rio Grande River at Big Bend National Park [65].

The high quantity of benzo(a)pyrene in our mosquitofish sample from site 9 is of concern because quantities this high are very unusual in fish; the carcinogenic PAHs usually are broken down quickly in the liver [40]. Analyses of PAHs in fish tissues often show only traces of PAHs even when the sediments contain high concentrations of these compounds [70].

Greatly elevated concentrations of PAHs in fish or sediments can be indicative of localized contaminant hot spots, with much lower concentrations a half mile downstream (Brian Cain, personal communication). Environmental degradation of PAHs such as fluorene can be reduced by low dissolved oxygen and low algal productivity [92]. Both conditions are common at our site 9, where the highest concentrations of PAHs were found.

At all sites where total PAHs and heavy PAHs were elevated, there were also notable elevations of phenanthrene, fluoranthene, and pyrene, all lower molecular weight PAHs which exhibit significant acute toxicity and increased toxicity in sunlight [40].

Table 2. Residues (mg/kg) of polycyclic aromatic hydrocarbons in whole-body samples of mosquitofish.

Site Number Location Code	10 5W	9 12B	6 WOVC	17 MTC	7 GH	3 OOD	2 VSB	15 EAP	1 MC
naphthalene	0.01	0.19	ND	ND	ND	ND	ND	0.01	ND
fluorene	ND	0.50	ND	0.01	ND	ND	ND	ND	ND
phenanthrene	0.10	7.2	ND	ND	0.02	0.04	0.02	0.06	ND
anthracene	ND	1.1	ND	ND	0.01	ND	ND	0.03	ND
fluoranthrene	0.01	10.	ND	ND	ND	0.01	0.01	0.03	ND
pyrene	ND	8.7	ND	0.02	ND	0.01	ND	0.03	ND
1, 2-benzanthracene	ND	5.3	ND	ND	ND	ND	ND	0.02	ND
chrysene	ND	4.9	ND	ND	ND	ND	ND	0.26	ND
benzo(b)fluoranthrene	ND	3.5	ND	ND	ND	ND	ND	0.01	ND
benzo(k)fluoranthrene	ND	3.2	ND	ND	ND	ND	ND	ND	ND
benzo(e)pyrene	ND	1.2	ND	ND	ND	ND	ND	ND	0.01
benzo(a)pyrene	ND	5.3	ND	ND	ND	ND	ND	0.01	ND
1,2,5,6-dibenzanthracene	ND	1.6	ND	ND	ND	ND	ND	ND	ND
benzo(g,h,i)perylene	ND	8.1	ND	ND	ND	ND	ND	0.01	0.01
Weight (g)	22	44	52	50	85	135	75	96	83
Moisture (%)	78.0	92.0	78.0	78.0	77.0	90.2	83.0	77.0	79.0
Lipid (%)	2.95	2.10	2.45	2.00	3.58	1.06	2.26	0.91	1.93

Aliphatic Hydrocarbons

Aliphatic hydrocarbons are a component of motor oil and other petroleum products. Like polycyclic aromatic hydrocarbons (PAHs), high aliphatic concentrations can be a clue that oil or petroleum pollution may be present. They are also present in sewage [69], urban runoff [69], and municipal landfill leachates [80,85]. Some of these occurrences are probably the result of contamination of these mediums by petroleum products. Low levels of aliphatics also occur naturally [68,69].

Aliphatics tend to be less toxic than PAH's (Brian Cain, U.S. Fish and Wildlife Service, personal communication). However, when exposed to chlorine, some aliphatics undergo haloform reactions to form trihalomethanes (THMs) [101]. The carcinogenic and teratogenic compound chloroform is most common THM produced by chlorination of sewage effluents and drinking water [8,102]. This potential problem is relevant to the Trinity River due to the large amount of chlorine which has entering the river from large sewage treatment plants (see detailed discussion in section on chlorine impacts).

Due to cost, aliphatics were analyzed in only six Trinity River samples. Four of the samples were from site 18, where a slick of oil, presumably used motor oil from a downtown Fort Worth storm drain, was on the surface of the water at the time of our collections. Every aliphatic in our scan was detected above the 0.01 mg/kg detection limit in all of the samples from site 18. Included in the aliphatic scan were n-dodecane, n-tridecane, n-tetradecane, octylcyclohexane, n-pentadecane, nonylcyclohexane, n-hexadecane, n-heptadecane, pristane, n-octadecane, phytane, n-nonadecane, and n-eicosane. All of these aliphatics are common components of used motor oil [75].

The other two samples, mosquitofish samples from sites 1 and 3, had very similar, low levels of aliphatics. Octylcylohexane, nonylcyclohexane, and n-eicosane were below detection in both samples, and all values were below 0.08 mg/kg except for n-heptadecane, which was 0.72 mg/kg at site 1 and 0.69 mg/kg at site 3.

The lowest values for all aliphatics at the oil spill site (site 18) were from spiny softshell turtles, the highest from redfin shiners, and intermediate levels were found in composite fatty tissue samples dissected from Texas cooter turtles and red-eared turtles.

Samples from site 18 with concentrations above 1 mg/kg aliphatics included redfin shiners, (1.15 mg/kg phytane and 5.71 mg/kg n-heptadecane); fat from Texas cooter turtles (2.6 mg/kg n-heptadecane); and fat from red-eared turtles (1.3 mg/kg n-heptadecane).

In contrast to the results of our recent study of the Rio Grande River at Big Bend National Park [65], Trinity

River samples more frequently showed a predominance of even-numbered carbon chains over odd-numbered carbon chain n-alkanes. According to some authors, this would indicate less likelihood that the aliphatics were from natural sources [68,69]. However, other authors have stated that this ratio theory does not appear to be predictive of sources in bird tissues [128]. The theory also did not appear to be uniformly predictive of apparent origins of aliphatics (for example, at the oil spill location, site 18) in fish and turtles we collected in the Trinity River. Therefore, our data does not provide an example of the utility of this theory for predicting origins of aliphatics in the fresh waters of Texas.

METALLIC CONTAMINANTS

Aluminum

Aluminum has been implicated as a neurotoxic agent in a number of studies [130,131,134]. A primary mechanism for aluminum-induced toxicity is free-ion aluminum ($Al^{3}+$) substitution for magnesium at critical enzyme sites and resultant depressions in magnesium-dependent functions [133]. Aluminum concentrations in living organisms tend to be low, but citrate or other acidic mediums can increase aluminum uptake [132]. Much research is now being undertaken to determine how much of a role aluminum plays a role in neurological problems such as Alzheimer's disease [130]. Aluminum chloride produces chromosomal aberrations in bone marrow of miçe and is mutagenic to plant chromosomes [61]. Aluminum is also one of the few contaminants which accumulates in fish muscle [57].

Since aluminum is a common component of soils and sediments, it is more prone to gut-content bias of whole-body samples than most trace elements [19]. Other sources of aluminum include treated drinking water, baking soda, and food additives [132].

Aluminum in water and sediments is much more toxic to fish when mobilized by low pH [122]. This may be a significant factor in the Trinity River, since we found pockets of low pH in areas of industrial discharges or chlorine leaks (see section on fish kills). Low levels (0.10 to 0.15 mg/L) of monomeric aluminum in water çan be toxic to striped bass larvae at pH levels of 6.9 to 7.3 [118]. At the time of our collections, many of our sites had pH levels below 7.3 (see section on pH).

Apparently, few studies have been done on aluminum concentrations in the water or sediments of the Trinity River. The few which have been conducted show considerable amounts of total aluminum in river water downstream of Dallas (Jack Davis, Texas Water Commission, personal communication). The latest EPA water quality criteria for aluminum states that when pH is between 6.5 and 9.0, aluminum should not exceed 87 ug/L more than once every three years [122], so EPA considers low levels of aluminum to be potentially toxic.

Aluminum was detected in all 77 Trinity River samples analyzed for metals, ranging from 1.3 mg/kg in snapping turtle muscle from site 15 to 1010 mg/kg in whole-body mosquitofish from site 9 just below downtown Dallas.

Gradient Monitoring Levels: In mosquitofish, aluminum showed a tendency to increase from upstream of Fort Worth to the South Loop 12 area just downstream of downtown Dallas (Fig. 9). A group of mosquitofish samples from sites 9, 10, 11, and 12 just downstream of Dallas had significantly higher concentrations of aluminum than a group of mosquitofish samples from sites (1, 16, and 27) upstream of Fort Worth or Dallas.

In a separate analysis, mosquitofish body burdens of aluminum were significantly higher from all sites having substantial treated sewage than from other sites.

High levels of aluminum have been reported in sludges from Fort Worth's Village Creek Sewage Treatment Plant. Aluminum sulfate (alum) is one source of aluminum which has been used extensively at drinking water treatment plants and is discharged with their filter back wash to sewage treatment plants (Richard Browning, Trinity River Authority, personal communication).

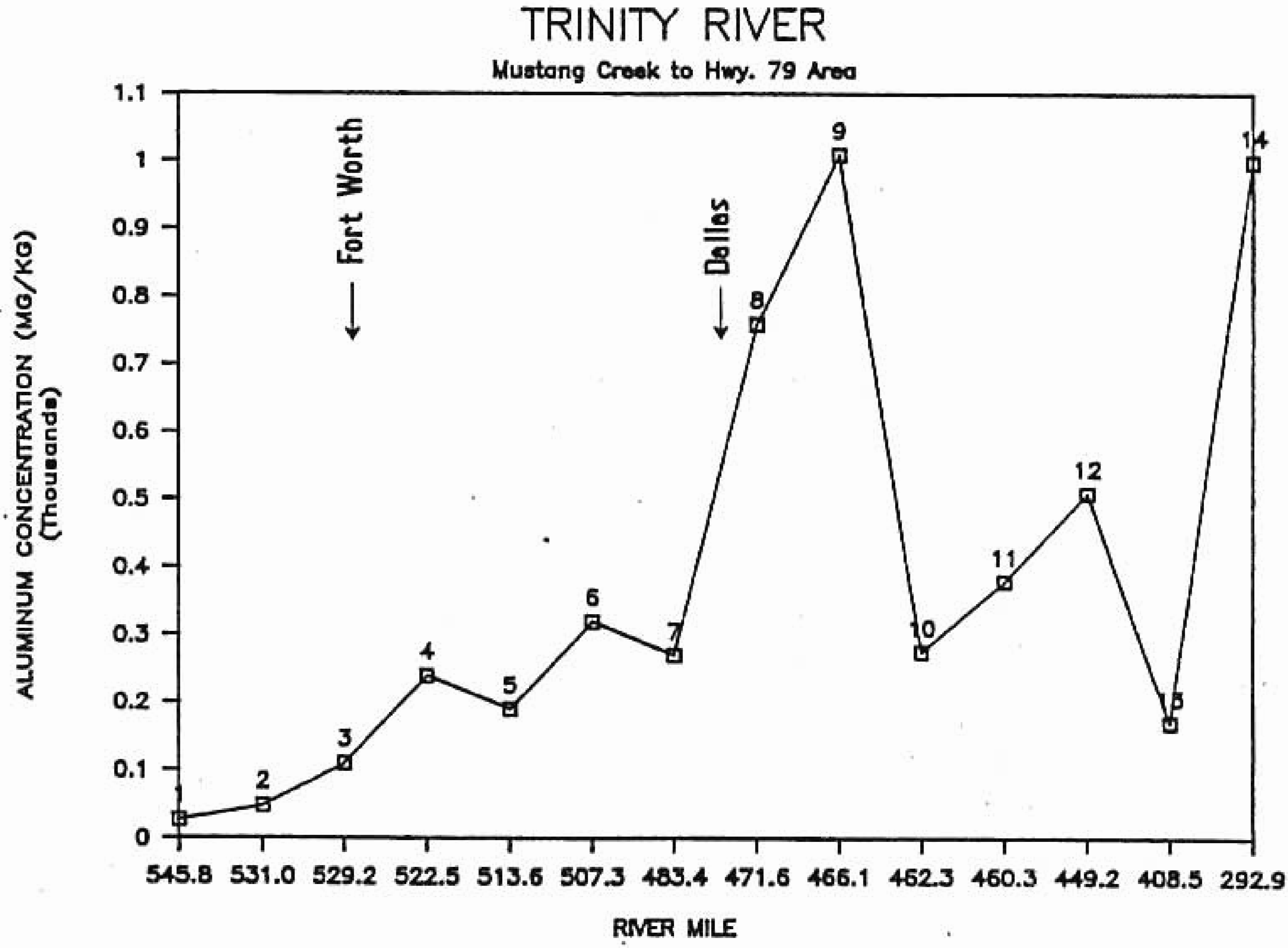


Fig. 9. Wet-weight concentrations of aluminum in whole-body samples of mosquitofish by river mile (site numbers are printed above each point).

Arsenic

Arsenic is one of the few metals which tends to concentrate in axial muscles of fish [29]. Arsenic is therefore of interest to those concerned with human health issues, since fillets are mostly muscle tissue. Arsenic acts as a cumulative poison [83] and is listed by the Environmental Protection Agency as one of 65 priority pollutants [58]. Arsenic is also listed among the 25 hazardous substances thought to pose the most significant potential threat to human health at priority superfund sites [93]. Recent reviews indicate arsenic has been associated with carcinogenic, mutagenic, and teratogenic impacts [21,129].

Arsenic enters rivers from air pollution (fossil fuel combustion) and soil erosion as well as from pesticides and industrial sources. Significant amounts of arsenic are known to leach from municipal landfills [46]. Pesticides are an additional source of arsenic in water [57].

Due to cost, we analyzed for arsenic in only 50 Trinity River samples. Arsenic was found above the detection limit (0.05 mg/kg) in all but 7 of these samples.

Predator Protection Level: Arsenic whole-body levels above 0.5 mg/kg are considered to be harmful to fish and predators [20]. All four Trinity River samples above the 0.5 mg/kg level were clam flesh samples rather than fish, including one sample (0.93 mg/kg) of unionid clams from site 14 and Asian clams from sites 14, 26, and 5 (0.72 to 0.89 mg/kg). Clams were not found at the most polluted sites below Dallas. These high levels for clams tend to confirm previous observations that clams, unlike fish, are efficient arsenic accumulators [57,83,95]. A nationwide study of arsenic in bivalves showed less variation in levels from various stations than was found for most other contaminants, with greater variation between different bivalve species from the same location [62].

Mean NCBP Levels: The geometric mean of whole-body concentrations of fish in a 1980-1981 national survey was 0.14 mg/kg arsenic [23], a level exceeded in 24 of 50 Trinity River samples. Included were numerous species of fish and turtles from both upstream and downstream sites. However, since this group of samples included a variety of turtle samples, it is not directly comparable to the NCBP means for fish only.

Gradient Monitoring Levels: Elevated concentrations of arsenic (above recommended criteria) in water and sediments have previously been reported for an area downstream of Dallas [42,71]. However, another summary seemed to suggest that arsenic may not be as highly elevated in sediments of the upper Trinity as are several other heavy metals [7].

In our study, plots and statistical analyses of arsenic levels in mosquitofish versus river miles, location groups, and runoff types revealed no clear trends or correlations. Certain clam species may be better than mosquitofish as indicator species for gradient monitoring of arsenic

Although most Trinity River tissue samples do not show highly elevated levels, arsenic is a compound for which we need more data to assess risks to fish and wildlife. A zero level of arsenic would be most effective at protecting from carcinogenic risk [21]. However, a zero level is probably not currently attainable due to the many potential sources of arsenic in the river.

Beryllium

Beryllium is a highly toxic, gray-white metal widely used in space technology, x-ray tubes, computer parts, and inertial guidance systems. Beryllium is listed by the Environmental Protection Agency as one of 65 priority pollutants [58], and is considered one of the 14 most noxious heavy metals [83]. Beryllium is also listed among the 25 hazardous substances thought to pose the most significant potential threat to human health at priority superfund sites [93]. Beryllium has been shown to be a carcinogen in rats and rabbits, to be teratogenic in a snail, and to cause developmental problems in salamanders [22].

Little is known about the effects of predators consuming prey carrying elevated concentrations of beryllium. We were unable to locate any alert or action levels pertaining to beryllium.

Primary sources of beryllium to a river are typically atmospheric fallout from the burning of coal, soil erosion, industrial discharges, and sewage treatment plants [22].
Floodplain-located landfills containing discarded pieces of high technology equipment might also be a potential source.

Beryllium was found above detection limits (0.002 mg/kg) in 56 of 77 Trinity River samples analyzed for metals.

Maximum Levels: The three highest concentrations of beryllium (0.033 to 0.052 mg/kg) were in mosquitofish downstream of Dallas, as were 6 of the 8 highest levels.

Gradient Monitoring Levels: Beryllium showed a tendency to increase from upstream to downstream in mosquitofish (Fig. 10). A group of mosquitofish samples from sites 9, 10, 11, and 12 just downstream of Dallas had significantly higher concentrations of beryllium than a group of mosquitofish samples from sites (1, 16, and 27) upstream of Fort Worth or Dallas.

Cadmium

Cadmium is very toxic to a variety of species of fish and wildlife. Cadmium causes behavior, growth, and physiological problems in aquatic life at sublethal concentrations [57]. Cadmium tends to bioaccumulate in fish [57], clams [90,95], and algae [95], especially in species living in close proximity to sediments contaminated by cadmium [95]. Cadmium acts as a cumulative poison [83] and is listed by the Environmental Protection Agency as one of 65 priority pollutants [58]. Cadmium is also listed among the 25 hazardous substances thought to pose the most significant potential threat to human health at priority superfund sites [93].

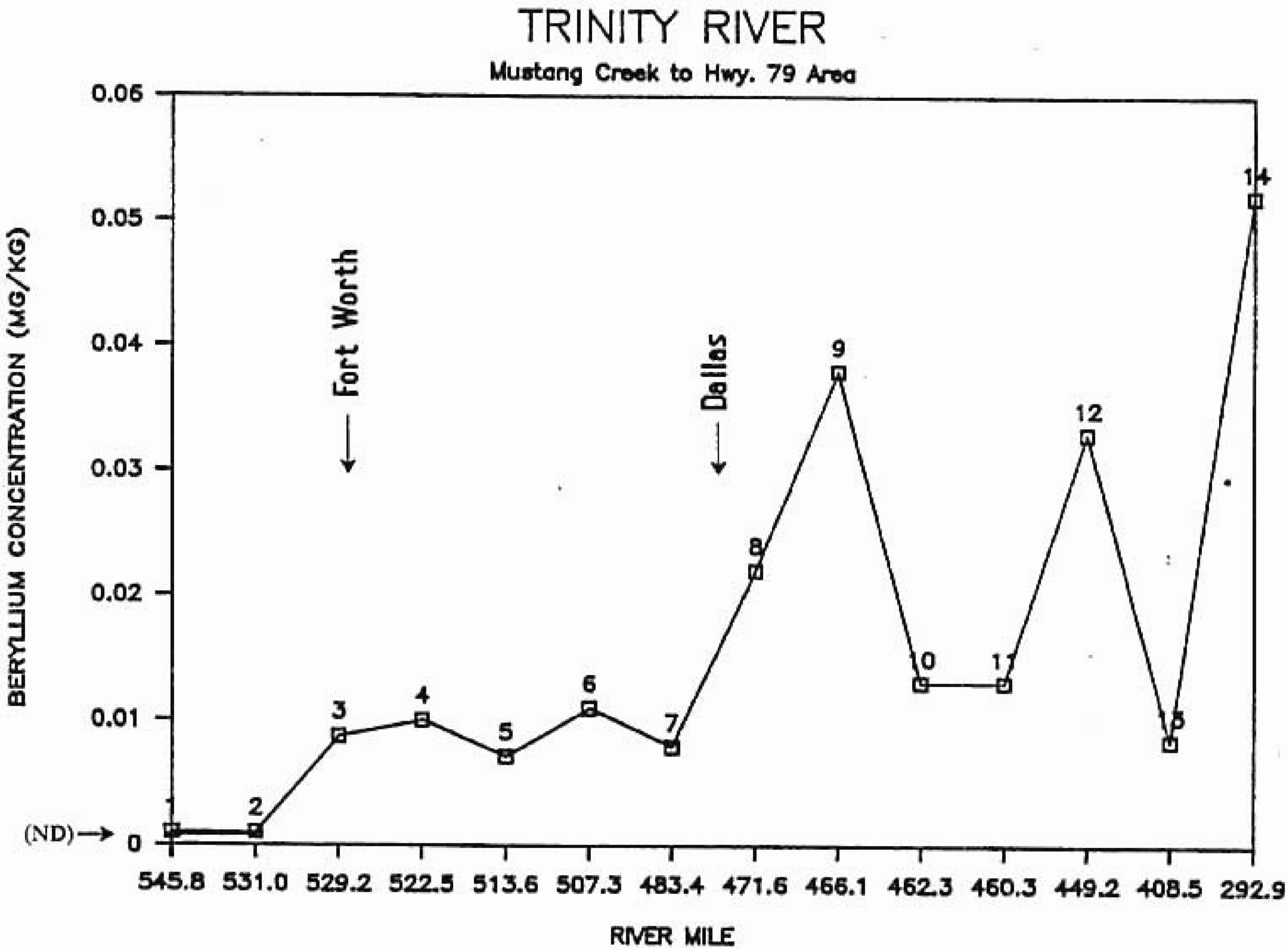


Fig. 10. Wet-weight concentrations of beryllium in whole-body samples of mosquitofish by river mile (site numbers are printed above each point, ND=statistical convention recorded for those values below the detection limit).

Cadmium is also a suspected carcinogen and has been shown to cause birth defects in mammals [61]. Mammals and birds consuming cadmium-contaminated food have experienced lowered sperm counts,kidney damage, increased mortality of young, elevated blood, sugar, and anemia [57].

Air pollution sources of cadmium include smelters, incinerators, oil furnaces, and coal combustion. Metal platers, scrap yards, batteries, television tubes, solar cells, fungicides, and various industrial discharges constitute additional sources [57]. In some localities, significant amounts of cadmium are also present in sewage sludges [61,94] and in leachates from municipal landfills [46,80]. The national average concentration for cadmium in U.S. soils is 5 mg/kg [98].

Cadmium concentrations above the detection limit (0.01 mg/kg) were found in 67 of 77 Trinity River samples analyzed for metals.

Predator Protection Level: Cadmium whole-body levels above 0.5 mg/kg are considered to be harmful to fish and predators [20]. Two Trinity River samples, a fatty tissue sample (0.65 mg/kg) from a composite of three Mississippi map turtles from site 11, and a composite whole-body sample of mosquitofish (0.71 mg/kg) from site 20, exceeded that level. Reproductive problems in fish may occur when tissue concentrations exceed 0.1 mg/kg [57].

Mean NCBP Levels: The geometric mean of whole-body cadmium concentrations in fish in a 1980–1981 national survey was 0.03 mg/kg [23], a level exceeded in 24 of 77 Trinity River samples. Included in this group were a large variety of fish, turtle, and clam species. However, since this group included a variety of non-fish samples, it is not directly comparable to the NCBP means for fish only. None of the samples above 0.03 mg/kg were from the control/reference site.

Gradient Monitoring Levels: Cadmium showed a general tendency to increase from upstream to downstream in mosquitofish (Fig. 11). The same trend was shown in sediment samples in a 1977 study by the Texas Water Quality Board. Elevated concentrations of cadmium have been found in sediments from downstream of Dallas [42,74]. Sediments from Beltline Road (6.5 miles downstream of our site 11) were 12.0 mg/kg, the highest recorded in the State at that time [74]. The highest levels of cadmium (7.0 to 12.0 mg/kg) were found in sediment samples from sites 9 through 12 (using our site numbers), with much lower concentrations upstream [74].

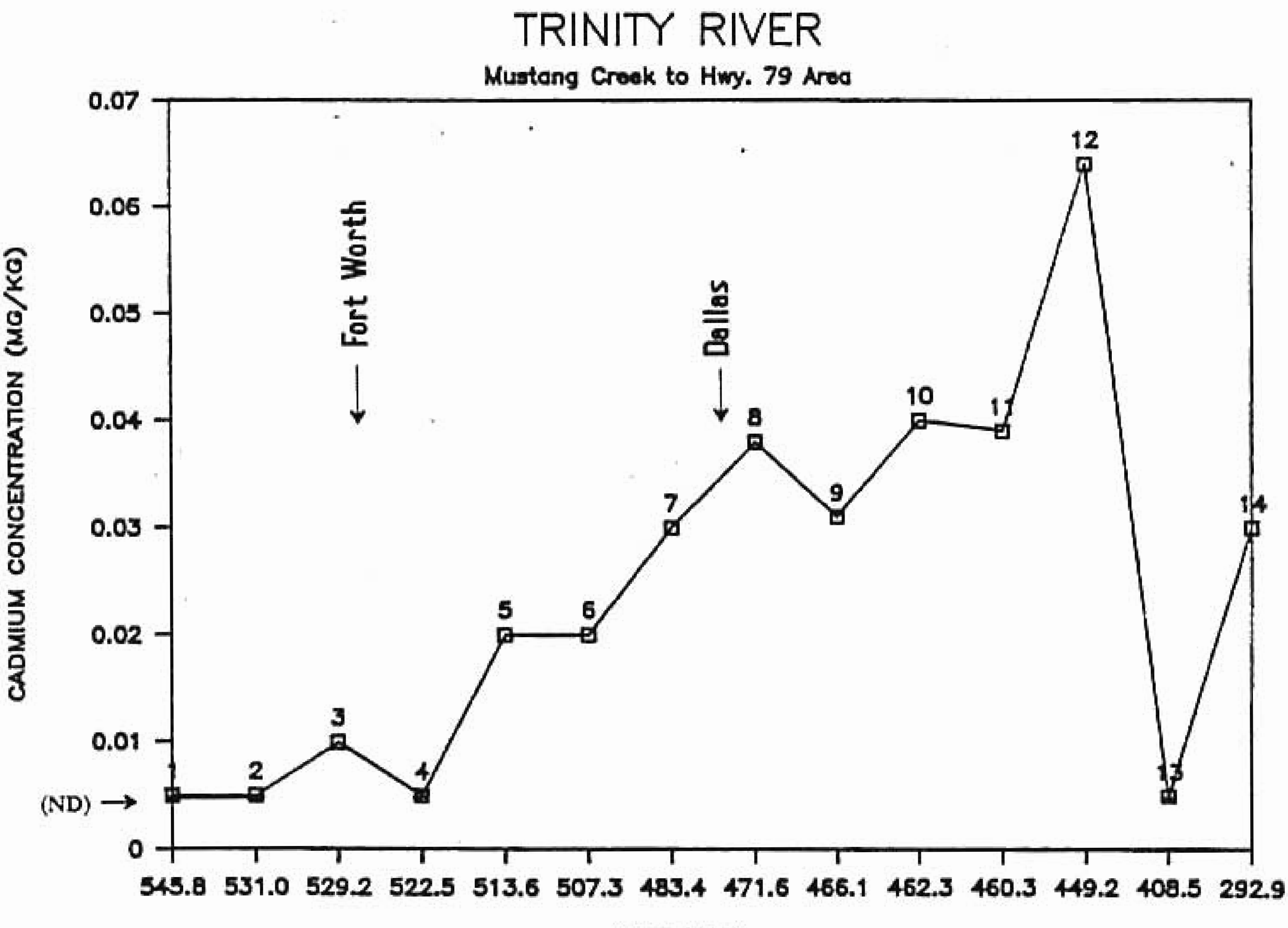


Fig. 11. Wet-weight concentrations of cadmium in whole-body samples of mosquitofish by river mile (site numbers are printed above each point, ND=statistical convention recorded for those values below the detection limit).

Sediment concentrations of cadmium from our sites 9 through 12 exceeded the statewide 90th percentile level, 3.0 mg/kg, in at least 50% of the historical records from 1974 to 1985 [7]. Cadmium has also been reported to have been highly elevated in sediments as far south as highway 31 near Trinidad [91]. A cycle of biomobilization of sedimentary cadmium by algae, followed by movement of the algae downstream and return of the cadmium to the sediments when the algae dies, may play a role in moving cadmium downstream [95].

In our study, the two highest concentrations of cadmium in mosquitofish were both from suburban creek sites (sites 17 and 20). Cadmium was the only contaminant for which this was true.

Chromium

Chromium is a metallic element which is listed by the Environmental Protection Agency as one of 65 priority pollutants [58]. Chromium is considered one of the 14 most noxious heavy metals [83]. Chromium is also listed among the 25 hazardous substances thought to pose the most significant potential threat to human health at priority superfund sites [93].

Trivalent and hexavalent forms of chromium are the most significant from the standpoint of potential impacts to fish and wildlife [24,57]. Since the valence states are subject to change, we analyze tissues for total chromium. During the laboratory digestion of tissue samples, most chromium is changed to the trivalent form.

Known sources of chromium include metal platers and a wide variety of chemical, photography, metal plating, scrap metal, machine shop, power plant, and industrial facilities [24,57]. Elevated chromium levels have been found in some samples of sewage sludge from the Dallas/Fort Worth area (Ron Carlson, City of Fort Worth, personal communication). Chromium is also present in the leachate of some municipal landfills [80].

Chromium concentrations above the detection limit (0.20 mg/kg) were found in 69 of 77 Trinity River samples. Maximum Levels: The two highest concentrations of chromium were a fatty tissue sample (6.0 mg/kg) from a composite of three Mississippi map turtles from site 11 and a composite whole-body sample of mosquitofish (9.7 mg/kg) from site 23. Site 23 was a Fort Worth storm drain site receiving runoff from a large metal scrap yard.

Other samples above 2.0 mg/kg chromium included a crayfish sample from site 5, a composite sample of turtle fat from 5 Texas cooter (turtles) from site 18, and a composite sample of asiatic clam tissue from site 5. Chromium has a higher bioaccumlative potential in clams and crayfish than in fish [83].

A survey of Pennsylvania fish from 16 sites revealed detectable chromium at 4 sites, with whole-body concentrations of chromium ranging from 0.1 to 0.26 mg/kg [57]. Based on our review of data from several Fish and Wildlife Service studies in the southwest, we consider chromium levels above 0.8 mg/kg in fish and wildlife tissues to be definitely elevated levels [65,78,79]. That level was exceeded by 28 of 77 Trinity River samples.

Predator Protection Level: Apparently the only chromium level which has been proposed as a protective standard for animal tissues is 0.20 mg/kg [24], a level exceeded by 69 of 77 Trinity River samples. All 8 of the Trinity River samples which were below this concentration were located upstream of major urban areas.

Gradient Monitoring Levels: Chromium showed a tendency to increase from upstream to downstream in Trinity River mosquitofish (Fig. 12).

Mosquitofish from urban areas of the upper Trinity River had concentrations of chromium ranging from 0.2 to 1.7 mg/kg. Mosquitofish from rural sites on the Rio Grande River at Big Bend National Park had significantly lower concentrations of chromium than mosquitofish from the urbanized upper Trinity River [65]. Chromium concentrations in Big Bend National Park mosquitofish ranged from 0.14 to 0.54 mg/kg [65].

In a previous study by the Texas Water Quality Board, the chromium level in sediments from Beltline Road (6.5 miles downstream of our site 11) was 140 mg/kg, the highest recorded in the State at that time [74]. The highest levels of chromium (53.0 to 140.0 mg/kg) were found in sediment samples from sites 8 through 12 (using our site numbers), with much lower concentrations upstream [74]. Chromium concentrations in water from the area were also elevated [71]. Sediment concentrations of chromium from our sites 9 through 12 exceeded the statewide 90th percentile level, 72.1 mg/kg, in at least 50% of the historical records from 1974 to 1985 [7].

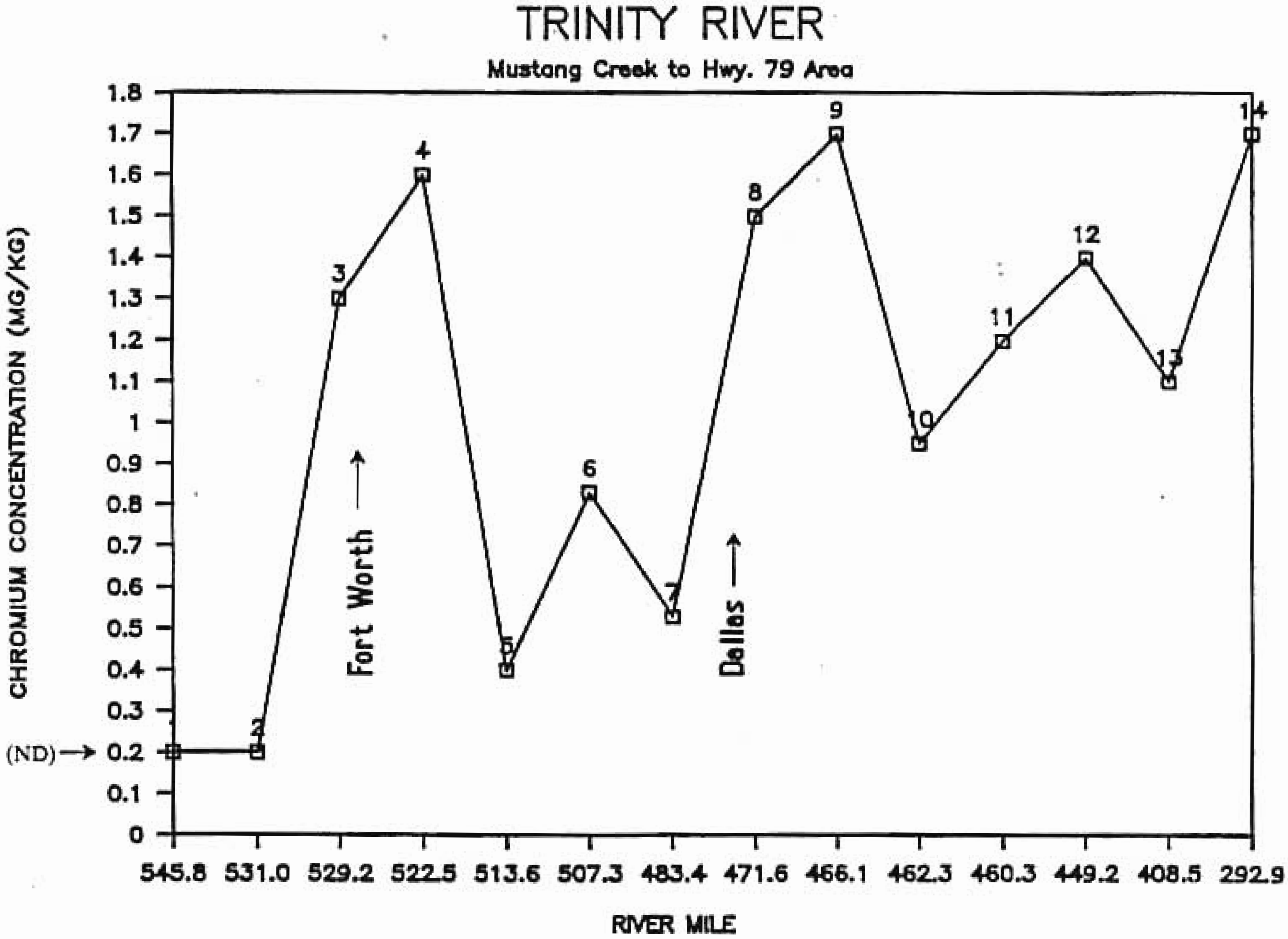


Fig. 12. Wet-weight concentrations of chromium in whole-body samples of mosquitofish by river mile (site numbers are printed above each point).

Copper

Copper is a commonly used metal which is listed by the Environmental Protection Agency as one of 65 priority pollutants [58]. Although copper is an essential dietary element for some plants and animals, it can be toxic to fish [25]. Some researchers believe that negative effects of copper on fish are more likely the result of toxicity of high concentrations in water than toxicity from intake of prey containing copper [25]. However, other researchers have concluded that fish living or foraging in contaminated sediments may accumulate it directly from the sediments [95].

Copper is one of the most common contaminants associated with urban runoff, and specific sources include soil erosion, corrosion of pipes and tubes, industrial discharges, and sewage treatment plant discharges [25]. Copper is also present in the leachate of some municipal landfills [80] and in sludges generated by sewage treatment plants [94]. The national average concentration for copper in U.S. soils is 30 mg/kg [98].

In water, copper acts synergistically with other common urban contaminants such as ammonia, cadmium, mercury, and zinc to produce an increased toxic effect on fish [26,47]. Sublethal concentrations adversely affect minnow fry survival and growth [57].

Copper concentrations above the detection limit (0.01 mg/kg) were found in 74 of 77 Trinity River samples. The three samples containing less than the detection level were from red-eared turtle shells.

Maximum Levels: The highest copper concentrations were in whole-body samples of spiny softshell turtles from sites 18 (12.8 mg/kg) and 11 (18.5 mg/kg) and from crayfish from site 5 (25.4 mg/kg). The seven highest concentrations were from crayfish, turtles, and clams rather than fish.

Alert Levels: We know of no action levels or alert levels which have been proposed for concentrations of copper in tissues of fish and wildlife in the United States. The Australian National Health and Medical Research Council recommends 30 mg/kg copper as a maximum content for seafood products, and this is the only "standard" we have seen proposed for tissue concentrations of this contaminant [84].

Mean NCBP Levels: Copper whole-body levels above 0.9 mg/kg are higher than 85% of all fish in a national survey [23]. This level was exceeded in 64 of 77 Trinity River samples. However, our Trinity River samples included a variety of turtle and invertebrate samples and is therefore not directly comparable to the NCBP means for fish only.

Gradient Monitoring Levels: Our mosquitofish data showed no uniform upstream/downstream distribution trends for copper. If they had been more uniformly present, clams might be a better choice for gradient monitoring of copper since they have an affinity for heavy metals and tend to be good indicators of metal pollution in general [95]. A nationwide study of copper in bivalves showed less variation in levels from various locations than from various species [62]. Nevertheless, copper concentrations in the lean tissues of mosquitofish, bullhead minnows, and red-eared slider turtles were at least slightly higher at the site impacted by urban runoff and urban point sources (site 11) than those from our reference/control site (site 1).

In a previous study by the Texas Water Quality Board, copper levels in sediments from Beltline Road (6.5 miles downstream of our site 11) were the highest recorded in the State at that time [74]. Copper concentrations in water from this area were above recommended Environmental Protection Agency water quality criteria [71], so elevated copper levels may be influencing the distribution of aquatic life. The number of insect and macroinvertebrate species is very sensitive to the degree of exposure to elevated levels of copper [110,111].

Sediment concentrations of copper from our sites 9 through 12 downstream of Dallas exceeded the statewide 90th percentile level, 40.0 mg/kg, in at least 50% of the historical records from 1974 to 1985 [7]. A cycle of biomobilization of sedimentary copper by algae, followed by movement of the algae downstream and return of the copper to the sediments when the algae dies, may play a role in moving copper downstream [95].

Iron

Little is known about the effects of predators consuming fish carrying excess iron. Iron tends to accumulate in the brains of rats as they age and may play a role in oxidative damage to brain tissues [123].

At all three Trinity River sites sampled for water during the July fish kill of 1985, iron was found to be elevated above levels which the Environmental Protection Agency recommends not be exceeded at any time, [56]. The Texas Parks and Wildlife Department concluded that flocculation of iron may have contributed to that kill by removing dissolved oxygen from the water and/or coating the gills of fish [56].

The primary sources of iron in rivers include soil erosion,urban runoff, and industrial discharges. Iron is also present in the leachate of some municipal landfills [80].

Iron was found above detection limits in all 77 Trinity River samples.

Maximum Level: The highest level of iron (1820 mg/kg) was in a fatty composite sample of three Mississippi map turtles from site 11. The 12 highest values (230–1820 mg/kg) were all from samples of turtles or mosquitofish from polluted areas.

Gradient Monitoring Levels: Iron showed a tendency to increase from upstream to downstream in mosquitofish (Fig. 13). A group of mosquitofish samples from sites 9, 10, 11, and 12 just downstream of Dallas had significantly higher concentrations of iron than a group of mosquitofish samples from sites (1, 16, and 27) upstream of Fort Worth or Dallas.

Mosquitofish from rural sites on the Rio Grande River at Big Bend National Park had iron concentrations ranging from 33 to 66 mg/kg [65]. These concentrations were lower than all but 4 of 24 mosquitofish from the Trinity River.

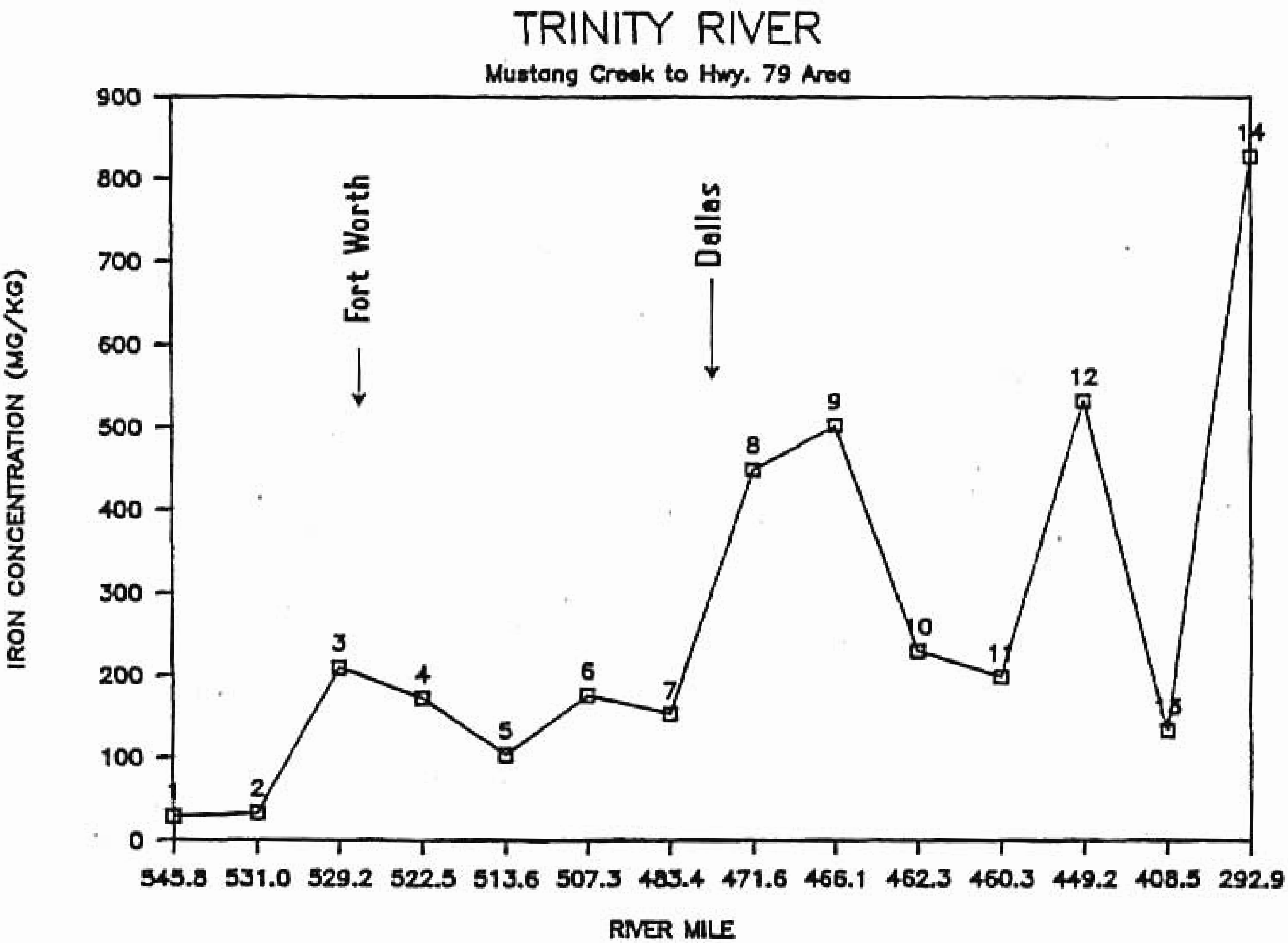


Fig. 13. Wet-weight concentrations of iron in whole-body samples of mosquitofish by river mile (site numbers are printed above each point).

Lead

Lead is a heavy metal which is very toxic to aquatic organisms, especially fish [57]. It tends to bioaccumulate in mussels and clams [90,95]. Benthic fish may accumulate lead directly from the sediments [95].

Lead functions as a cumulative poison [83] and is listed by the Environmental Protection Agency as one of 65 priority pollutants [58]. Lead is also listed among the 25 hazardous substances thought to pose the most significant potential threat to human health at priority superfund sites [93].

All measured effects of lead on living organisms are adverse, including those negatively affecting survival, growth, learning, reproduction, development, behavior, and metabolism [66]. Effects of sublethal concentrations of lead include increased mucous formation, delayed embryonic development, suppressed reproduction, inhibition of growth, and fin erosion [57].

Lead shot poisoning of waterfowl has been widely publicized but can also occur in bald eagles and other species of fish and wildlife [29]. The mosquitofish samples from site 15 were excluded from our whole-body metals analyses because the fish were found to contain lead shot in their stomachs.

Synergistic effects of lead and cadmium and additive effects of lead, mercury, copper, zinc, cadmium, and mercury have been documented for aquatic biota [29]. In birds, lead has also been implicated in decreases in eggshell thickness, growth, ovulation, and sperm formation [57]. The many negative effects of lead on physiology and heme formation [66] increase lead's potential for synergistic or additive effects with other contaminants and with low oxygen stress.

Typical sources of lead in rivers include atmospheric fallout from motor vehicle and smelter emissions as well as sewage sludge, batteries, pipes, lead shot, glazes, paints, and alloys. Significant amounts of lead are also known to leach from municipal landfills [46,80]. Lead is also a common contaminant in used motor oil [75] and in sludges generated by sewage treatment plants [94].

Airborne lead is deposited on vegetation and wildlife living near highways, and some urban zoos have experienced lead poisoning [28]. The national average concentration for lead in U.S. soils is 10 mg/kg [98], but much higher concentrations are common near busy highways [43]. Sediments can act as a sink for lead and a continuing source of lead after the original source has subsided [66].

Lead concentrations above the detection limit (0.05 mg/kg) were found in 71 of 77 Trinity River samples.

<u>Maximum Level</u>: The highest lead concentration, 7.2 mg/kg, was from a shell composite sample dissected from five red-eared turtles from site 18, a storm drain in downtown Fort Worth. The three highest levels (2.7 to 3.7 mg/kg) in fish were all samples of mosquitofish from storm drains in downtown Fort Worth.

<u>Concern Level for Human Food</u>: There is no FDA action level for lead in fish, but an edible tissue guideline often cited as an upper limit for lead in foods is 0.3 mg/kg [27,66], a level exceeded in 43 of the 77 Trinity River samples. Included in this group were a variety of fish, turtle, and clam species and tissue types, but all were from polluted sampling sites. However, most of the Trinity River samples (including all of our fish samples) were whole-body samples or other tissues which would be consumed by fish and wildlife to a greater extent than by humans, so the 0.3 mg/kg level would not necessarily be protective of fish and wildlife predators.

A few of our non-fish samples would be considered edible tissues and exceeded the 0.3 mg/kg level, including: one composite sample (0.37 mg/kg) of muscle tissue dissected from 18 red-eared turtles from site 11; one composite sample (0.68 mg/kg) of muscle tissue dissected from five red-eared turtles from site 18; and one composite sample (1.4 mg/kg) of flesh dissected from asiatic clams from site 5.

<u>Mean NCBP Levels</u>: The geometric mean of whole-body concentrations of lead in fish in a 1980-1981 national survey was 0.17 mg/kg [23], a level exceeded in 57 of 77 Trinity River samples. Included in this group were a variety of fish, turtle, and clam species. However, since this group of samples included a variety of non-fish samples, it is not directly comparable to the NCBP means for fish only. Most of the 20 Trinity River samples below 0.17 mg/kg were from the control/reference site or other relatively unpolluted areas.

<u>Gradient Monitoring Levels</u>: A river mile plot of mosquitofish whole-body values for lead showed peaks just downstream of Fort Worth and just downstream of Dallas (Fig. 14). An analysis of all Trinity River sites showed that 17 of 23 mosquitofish samples had concentrations equaling or exceeding 0.1 mg/kg lead. Concentrations of lead in 3 samples of mosquitofish from the Rio Grande River at Big Bend National Park were all less than 0.1 mg/kg lead [65].

An group of mosquitofish samples from sites 1, 16, and 27 (upstream sites on the Trinity River) had significantly lower concentrations of lead than a group of mosquitofish samples from all other Trinity River sites. A group of mosquitofish samples from sites 9, 10, 11, and 12 just downstream of Dallas had significantly higher concentrations of lead than a group of mosquitofish samples from sites (1, 16, and 27) upstream of Fort Worth or Dallas.

Sediment concentrations of lead from our sites 9 through 12 exceeded statewide 90th percentiles in at least 50% of the historical records from 1974 to 1985 [7]. These highly elevated levels were still present at our site 12 as late as October of 1985, after the collections for this report were made [91].

Elevated levels of lead have also been reported from Trinity River water samples. In water samples collected by Texas Parks and Wildlife Department from site 24 during the July fish kill of 1985, lead was found to be elevated above levels which the Environmental Protection Agency recommends not be exceeded [56].

Lead was one of the few contaminants for which turtle shells appeared to be efficient accumulators, a reflection of the fact that lead tends to be deposited in bone as a cumulative poison [26]. Like manganese, lead tends to be deposited in bone, skin, and scales to a much greater extent than in muscle tissue. Therefore fillets can be contaminated by common fish-cleaning techniques [27]. However, fish from polluted areas do build up substantial concentrations of lead in muscle tissue and whole-body analysis of fish for lead is still recommended for general environmental monitoring [27].

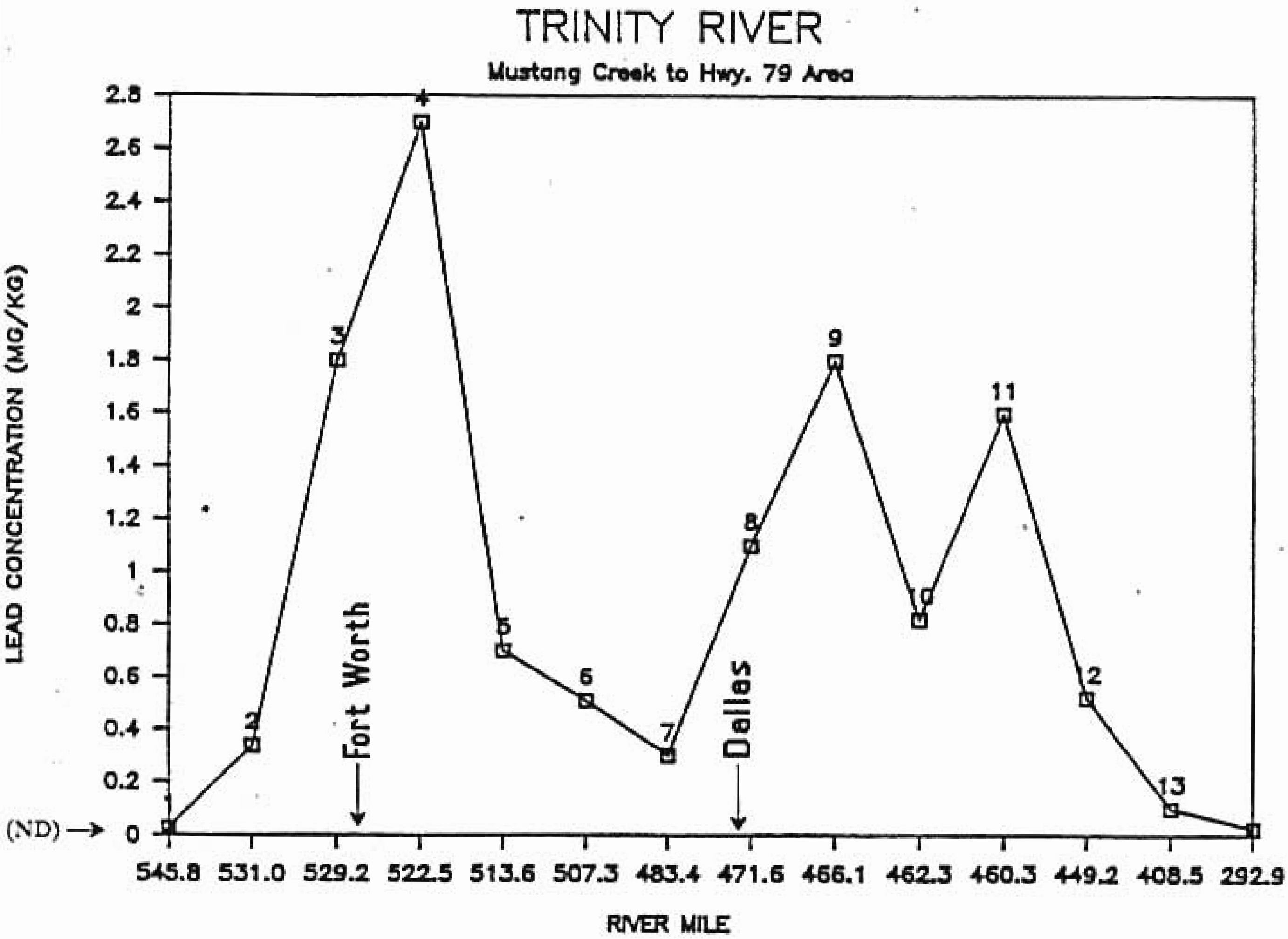


Fig. 14. Wet-weight concentrations of lead in whole-body samples of mosquitofish by river mile (site numbers are printed above each point, ND=statistical convention recorded for values below the detection limit).

Previous reports have downplayed the ability of lead to biomagnify or bioaccumulate to high levels in biota other than bivalves such as clams [30,66]. However, our data show that mosquitofish, softshell turtles, Texas cooter turtles, bullhead minnows, crayfish, and red-eared turtles accumulated significant amounts (>1.0 mg/kg) of lead in the Trinity River. Nevertheless, lead concentrations were not higher in top of the food-chain predators like gar than they were in mosquitofish.

Another recent report [53] documented that lead concentrations in crayfish and midges correlated with lead in effluent water and that exclusion of crayfish gut concentrations did not appreciably change the correlation.

Manganese

Manganese is a required trace element, and fish have some ability to excrete excess manganese, but the precise significance of excess body burdens of manganese is unclear for most species of fish and wildlife. Manganese tends to accumulate in bone, skin, and scales [27].

Manganese is thought to present less of a toxicity problem in natural waters than most of the other contaminants covered in this report [12]. However, some poisonings from excess levels have occurred in humans [57].

Manganese occurs naturally in surface waters from soil erosion. Other sources include air pollution deposition from power plants, sewage treatment plant effluents, and leachates from municipal landfills [80]. We have seen unpublished lab reports of elevated levels of manganese in ground water monitor wells for a municipal landfill in the Dallas/ Fort Worth area.

Manganese concentrations were detected in all 77 Trinity River samples.

Maximum Level: The highest manganese concentration, 754 mg/kg, was from a flesh composite sample dissected from unionid clams from site 14. Other concentrations above 22 mg/kg included Asian clam shells from sites 14 and 26 and mosquitofish from sites 17 and 22.

Gradient Monitoring Levels: An upstream group of mosquitofish samples from sites 1, 16, and 27 had significantly lower concentrations of manganese than a group of mosquitofish samples from all other sites. Mosquitofish samples from the rural Rio Grande River at Big Bend National Park had manganese concentrations ranging from 6.0 to 13 mg/kg, concentrations lower than most of the Trinity River samples of mosquitofish [65].

However, manganese was one of the few metals which was not consistently elevated downstream of Dallas compared to upstream of Fort Worth, in all other species of fish and wildlife collected (see discussion comparing reference/ control and impacted sites). Some aquatic organisms can apparently regulate the uptake of manganese [95].

Mercury

Mercury is a cumulative poison [83] and is the heavy metal most toxic to fish [33]. Mercury is listed by the Environmental Protection Agency as one of 65 priority pollutants [58].

Mercury is also one of the few metals which strongly bioconcentrates and biomagnifies; has only harmful effects with no useful physiological functions when present in fish and wildlife; is a carcinogen, mutagen, and teratogen; and is easily transformed from a less toxic inorganic form to a more toxic organic form in fish and wildlife tissues [33]. It is a metal whose use should be curtailed as much as possible to prevent impacts to fish and wildlife [33]. When exposed to mercury in both mediums, fish accumulate more mercury from sediments than from water [95]. Lower pH levels (indicating increased acidification) are correlated with increased mercury accumulation in fish [120].

Sources of mercury include batteries, vapor discharge lamps, thermometers, older-style seals in sewage treatment plants, sewage treatment plant discharges, the chloralkali industry, paints, pesticide compounds, switches, valves, dental labs and offices, pharmaceuticals, scientific and analytical laboratories, soil erosion, and air pollution deposition from fossil fuel combustion and smelters [33]. Leachates of municipal landfills contain mercury [80], possibly due to the disposal of items such as mercury batteries, thermometers, and electrical switches. Scrap metal dealers who accepted mercury and laboratories analyzing soil samples have been a significant source of mercury in Dallas/Fort Worth Storm Drains (Ross Muir, Tarrant County Health Department, personal communication). Contact lens solutions containing thimerosal are an additional source of small amounts of mercury. Many sources of small amounts of mercury can have a cumulative impact on a small river like the Trinity, due to mercury's persistence.

Mercury in bottom sediments is resuspended during floods and carried further downstream. Such events have resulted in increased levels of mercury in fish, as noted in a previous Fish and Wildlife Service study in Montana [32]. Mercury is one of the few metals which accumulates in the axial muscles of fish, so fillet levels are typically closer to whole-body concentrations than for most other contaminants [27].

Mercury concentrations above the detection limit (0.02 mg/kg) were found in 73 of 77 Trinity River samples.

Maximum Level: The highest mercury concentration, 0.85 mg/kg, was from a composite sample of fat dissected from three Mississippi map turtles from site 11. This was the only sample which exceeded the 0.5 mg/kg whole-body guideline previously proposed to avoid harm to fish, ducks,and predators [20,31]. For comparison, human health standards for fish have included a 1.0 mg/kg U.S. FDA standard and a 0.5 mg/kg Canadian standard [32]. A muscle tissue level of 0.232 mg/kg has been shown to cause decreased swimming ability in fish [57].

Predator Protection Level: The most recently recommended level for the protection of avian predators which consume fish and other aquatic organisms is that total mercury in these food items should not exceed 0.1 mg/kg [33]. The author believes the 0.1 mg/kg alert level may be inadequate to protect fish and wildlife, since concentrations of 0.1 mg/kg fed to ducks reduced fertility and inhibited food conversion [34]. The 0.1 mg/kg level was exceeded in 17 of 77 Trinity River samples. Included in this group were a variety of fish and turtles, all from areas just downstream of Dallas or other highly polluted sites. The three highest levels (0.19 to 0.23 mg/kg) exceeding 0.1 but not 0.5 mg/kg, were all in fish from sites just south of Dallas, including composite samples of mosquitofish from site 12, carp from site 11, and smallmouth buffalo fish from site 24.

Predator Alert Level: Due to mercury's potency, an argument could be made for applying an application factor to FDA's 1.0 mg/kg action level for mercury in fish used as human food. Assuming fish typically are eaten by humans at no more than 3 of 21 meals per week, and further assuming that fish usually account for no more than half of the food at each of those meals, a typical maximum percentage of fish in the human weekly diet could be estimated as 3 x 0.5 divided by 21 meals or 7.14% of the diet.

On the other hand, predators such as bass may be eating other fish and wildlife exclusively, and the tendency of contaminants like mercury to bioaccumulate in predators or biomagnify up the food chain make a lower standard necessary. Fish and wildlife predators usually consume the entire body of a prey species rather than fillets.

Concentrations of metal contaminants in muscle tissue are typically 0.5 to 0.6 of the concentration of a whole-body sample [63]. Dividing the 7.14% level by 2 to compensate for the difference between whole-body and muscle concentrations would yield a fish and wildlife application factor of 0.036. Multiplying 0.036 by the 1.0 FDA action level would yield an alert level of 0.036 mg/kg, a level exceeded by 64 of 77 Trinity River samples.

The 0.036 mg/kg level is not much lower than concentrations fed to chickens (0.050 mg/kg) which resulted in chickens concentrating mercury to levels high enough to be of concern to human consumers [33]. However, more work would have to be done to definitively develop a predator alert level for mercury, so we have provided this simple derivation for illustrative and discussion purposes rather than for regulatory purposes.

Gradient Monitoring Levels: In mosquitofish, mercury showed a tendency to increase from upstream of Fort Worth to downstream at Malloy Road Bridge south of Dallas (Fig. 15).

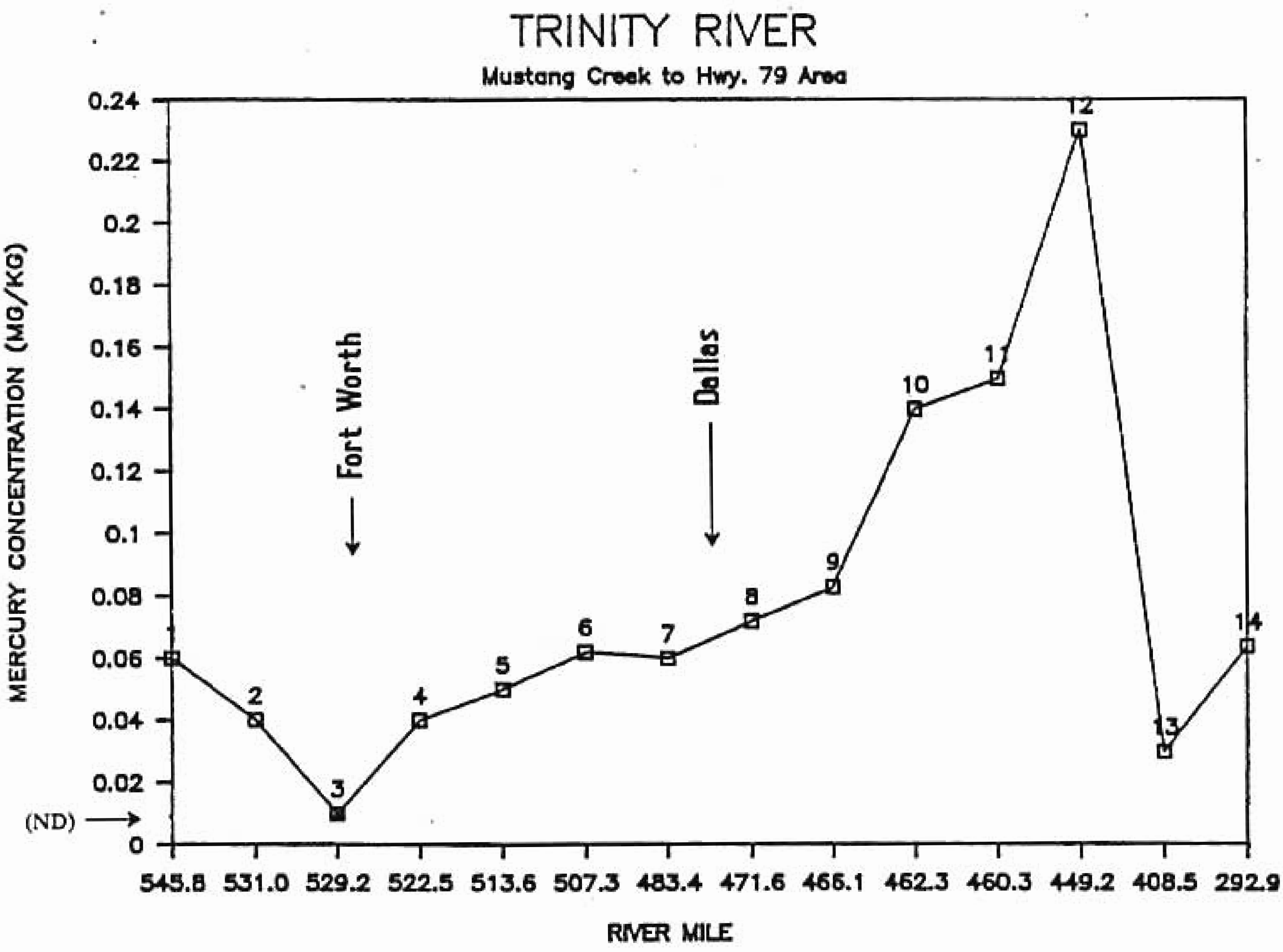


Fig. 15. Wet-weight concentrations of mercury in whole-body samples of mosquitofish by river mile (site numbers are printed above each point, ND=statistical convention recorded for values below the detection limit).

In a previous report, the Texas Parks and Wildlife Department reported that mercury was one of the few metals which appeared to increase in concentration as one progressed downstream [56].

In related findings, the Trinity River Authority indicated that high mercury concentrations were occasionally found at various stations downstream of Dallas, some quite a distance downstream [42]. The highest mercury level (2.318 mg/kg) was in shad muscle tissue from the Crockett area; the 0.5 mg/kg level was exceeded in 10 of 38 fish samples collected downstream of Dallas [42]. Algae may be playing a role in moving mercury downstream [95].

In a previous study by the Texas Water Quality Board, mercury levels in sediments from Beltline Road (6.5 miles downstream of our site 11) were the highest recorded in the State at that time [74]. Sediment concentrations of mercury from our site 12 exceeded the statewide 90th percentile level, 0.32 mg/kg, in 50% of the historical records from 1974 [7].

Mercury concentrations in water above recommended criteria have also been reported for the Trinity River [71]. At all three Trinity River stations sampled for water during the July fish kill of 1985, mercury was found to be elevated above levels which the Environmental Protection Agency recommends not be exceeded at any time [56].

In our study, the four highest mercury levels in mosquitofish were from sites 9, 10, 11, and 12 just downstream of Dallas. Mosquitofish samples from these four sites had significantly higher concentrations of mercury than a group of mosquitofish samples from sites (1, 16, and 27) upstream of Fort Worth or Dallas.

Except for 5 sites below Dallas, Trinity River mosquitofish concentrations of mercury (0.025 to 0.065 mg/kg) were lower than those recorded for mosquitofish from Big Bend National Park, an area where mercury mining has occurred in the past [65].

A similar trend was found in softshell turtles. Whole-body concentrations of 0.050 and 0.060 mg/kg mercury were found in softshell turtles from two comparatively rural Trinity River sites (sites 1 and 15). Concentrations of 0.18 mg/kg were found in whole-body samples of softshell turtles from two highly polluted sites (sites 11 and 18). The mercury level in one composite sample of softshell turtles from the Rio Grande River at Big Bend National Park was 0.073 mg/kg [65].

Nickel

Nickel is a metal which is abundant in the earth's crust. It is listed by the Environmental Protection Agency as one of 65 priority pollutants [58], and is considered to be one of the 14 most noxious heavy metals [83]. Nickel is also listed among the 25 hazardous substances thought to pose the most significant potential threat to human health at priority superfund sites [93].

Little information is available on the effects of nickel body burdens on fish and wildlife, but experimental doses of nickel have induced cancer in rats, guinea pigs, and rabbits [35]. Nickel is present in asbestos and may play a role in asbestos carcinogenicity [35]. Mixtures of nickel, copper, and zinc produced additive toxicity effects on rainbow trout [57].

Although nickel occurs naturally in rivers from soil erosion, it is usually elevated at least four times above background levels in most urban settings [35]. Sources include air pollution deposition from burning of fossil fuels, operation of motor vehicles, smelters, electroplating facilities, scrap yards, and various industrial sources [35]. Nickel is also a common contaminant in sludges generated by sewage treatment plants [94]. Nickel is also present in the leachate of some municipal landfills [80].

Nickel concentrations above the detection limit (0.02 mg/kg) were found in 60 of 77 Trinity River samples.

Maximum Level: The highest nickel concentration, 12 mg/kg, was from a composite sample of mosquitofish from site 25, a storm drain in downtown Fort Worth where a spill of nickel had occurred a year before our collections. This is a very high nickel concentration; the highest nickel concentration recorded in a survey of Pennsylvania fish from 14 sites was 0.41 mg/kg [57].

Concentrations above 0.9 mg/kg nickel appear to be elevated values in relationship to relatively unpolluted sites in the Southwest studied by our agency. Eleven of 77 Trinity River samples were above 0.9 mg/kg, including samples of Asian clam flesh, crayfish, mosquitofish, freshwater drum, longnose gar, and Mississippi map turtles. The clam and crayfish samples were from site 5 downstream of Fort Worth, and the other samples exceeding 0.9 mg/kg were from sites downstream of Dallas. Clams are generally better accumulators of nickel than fish [83,95].

Gradient Monitoring Levels: Nickel showed a tendency to increase from upstream to downstream in mosquitofish (Fig. 16). Sediment concentrations of nickel from our sites 9 through 12 exceeded statewide 90th percentiles in at least

50% of the historical records from 1974 to 1985 [7].

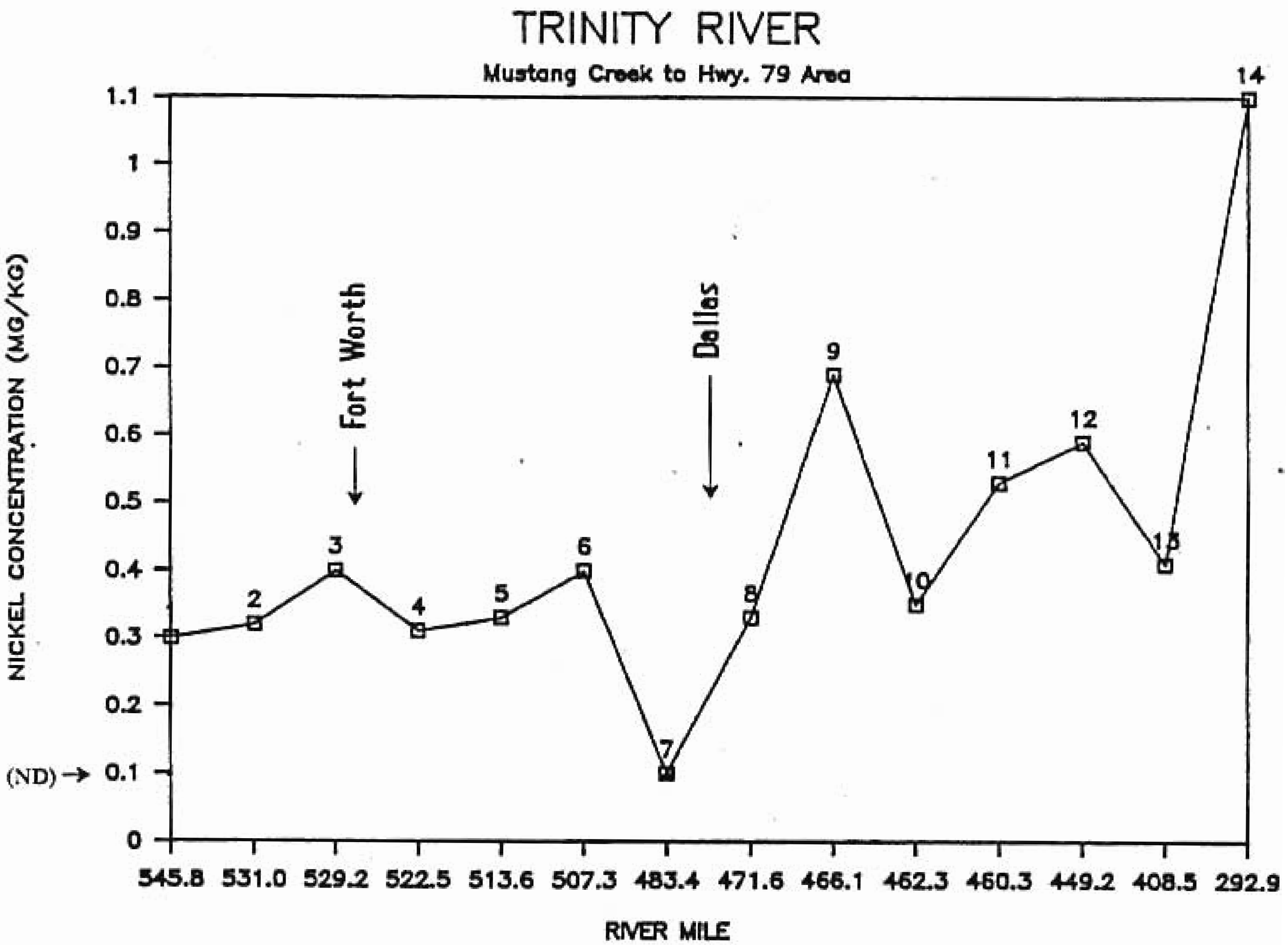


Fig. 16. Wet-weight concentrations of nickel in whole-body samples of mosquitofish by river mile (site numbers are printed above each point. ND=statistical convention recorded for values below the detection limit).

In a recent study in a rural area of Texas, we found concentrations of 0.05 to 0.21 mg/kg nickel in mosquitofish from the Rio Grande River at Big Bend National Park [65]. These concentrations were lower than all but 5 of 24 mosquitofish samples from the Trinity River.

Selenium

Selenium is one of the few metals which accumulates in the axial muscles of fish, so fillet levels are typically closer to whole-body concentrations than are most other contaminants [27].
Selenium also accumulates in the gonads of bass and bluegills [36] and has many teratogenic and toxic impacts upon fish and wildlife at high concentrations [37]. Selenium is listed by the Environmental Protection Agency as one of 65 priority pollutants [58].

The range between insufficient selenium in the diet of animals and too much is narrow, and the effects of either problem can be serious [63]. Effects may range from birth defects to sterility and death [63].

Other than areas impacted by agricultural drainage, very high concentrations of selenium in fish and wildlife occur primarily in areas where selenium is naturally high in the soils, where there is an influence of sewage sludge, or where coal fired power plants are present [37,44]. Man's uses of selenium include photocopying, glass manufacturing, the production of stainless steel, fungicides, lubricants, electronic devices, pigments, dyes, insecticides, and veterinary medicine [38,61]. A nationwide study of selenium in bivalves showed less variation in levels from various stations than was found for most other contaminants [62].

Due to cost, we analyzed selenium in only 50 Trinity River samples. Selenium was found above the detection limit (0.09 mg/kg) in all but one sample.

Maximum Level: The highest selenium concentration was 0.71 mg/kg. This concentration was found in a composite sample of unionid clam flesh from site 14.

Predator Protection Level: Selenium whole-body levels above 0.5 mg/kg are considered harmful to fish and predators [20], a level close to the geometric mean of 0.47 mg/kg for whole-body concentrations of fish in a 1980-1981 national survey [23]. The 0.5 mg/kg level was exceeded in 6 of 77 Trinity River samples. Included in this group were samples of Mississippi map turtles, mosquitofish, carp, spiny softshell turtles, and unionid clams, all from sites downstream of Dallas except for the mosquitofish, which were from site 20.

Gradient Monitoring Levels: The highest concentration of selenium in 24 samples of Trinity River mosquitofish was 0.52 mg/kg (site 20). For contrast, mosquitofish from a pond severely contaminated by agricultural drainage at Kesterson National Wildlife Refuge in California had selenium concentrations ranging from 26 to 31 mg/kg [77].

The selenium level in one composite sample of softshell turtles from the Rio Grande River at Big Bend National Park was 0.64 mg/kg [65]. This level is somewhat higher than those (0.26 to 0.43 mg/kg) found in softshell turtles from three sites (1, 15, 18) on the upper Trinity River. However, it is about the same (0.67 mg/kg) as the concentration recorded for a sample from highly polluted site 11 just downstream of Dallas [27].

Zinc

Zinc at low levels is an essential dietary element for animals and humans, but is toxic to fish at levels exceeding the minimum amount needed [57]. Fish, especially those living or foraging in sediments contaminated by zinc, may accumulate it directly from the sediments [95].

Little is known about potential impacts of excess body burdens of zinc on other species of fish and wildlife, except that in mammals excess zinc can cause copper deficiencies, affect iron metabolism, and interact with the chemical dynamics of lead and drugs [39]. Zinc is listed by the Environmental Protection Agency as one of 65 priority pollutants [58].

Zinc in water acts synergistically with copper and ammonia to produce an increased toxic effect on fish [26,47]. Elevated water concentrations of zinc have especially strong impacts on macroinvertebrates such as molluscs, crustaceans, odonates, and ephemeropterans [72]. A study in an Arkansas river system showed that macroinvertebrate concentrations were negatively correlated with zinc concentrations but not with concentrations of iron or copper [72]. These findings raise the possibility that zinc may be one of the many chemicals playing a role in fish and macroinvetebrate distribution in parts of the Trinity River.

Zinc is one of the most common contaminants associated with urban runoff. Other zinc sources include soil erosion, industrial discharges, pharmaceuticals, and pesticides [39]. In some areas up to 50% of the zinc comes from highway runoff [43]. The national average concentration for zinc in U.S. soils is 300 mg/kg [98]. Zinc is also present in the leachate of some municipal landfills [80] and is a common contaminant in sludges generated by sewage treatment plants [94].

Zinc was detected in all 77 Trinity River samples. Zinc whole-body levels above 40.1 mg/kg are higher than 85% of all fish in a national survey [23]. This level was exceeded in 24 of 77 Trinity River samples. Included in this group was a variety of fish, turtle, and clam species, from all different types of sites.

Maximum Levels: The highest zinc concentrations were from composite samples of unionid clam flesh from site 14 (87.5 mg/kg) and from turtle shells from sites 11, 15, and 18 (71.6 to 78.8 mg/kg). In a previous study by the Texas Water Quality Board, zinc levels in sediments from Beltline Road (6.5 miles downstream of our site 11) were the highest recorded in the State at that time [74].

Gradient Monitoring Levels: Sediment concentrations of zinc from our sites 9 through 12 exceeded the statewide 90th percentile level in 100% of the historical records from 1974 to 1985 [7]. These highly elevated levels were still present at our site 12 as late as October of 1985, after the collections for this report were made [91].

Elevated levels of zinc have also been reported from Trinity River water samples. In water samples collected by

Texas Parks and Wildlife Department from site 24 and State Highway 31 during the July fish kill of 1985, zinc was found to be elevated above levels which the Environmental Protection Agency recommends not be exceeded as a 24-hour concentration [56]. Zinc has found to be more acutely toxic to fish at higher temperatures than at lower temperatures [98]. This might be a factor in the Trinity River, where most fish kills have occurred during periods of elevated water temperatures.

However, in our study zinc was one of the few contaminants which was not consistently higher in fish and wildlife tissues downstream of Dallas than at our reference/control site (site 1) upstream of Fort Worth. It is possible that the fish and wildlife samples we collected were not particularly good indicators for measuring gradients of zinc. Zinc's role as a dietary requirement may be a factor. Some aquatic organisms can apparently regulate the uptake of zinc, and the bioavailability of zinc is related to sediment type [95]. A nationwide study of zinc in bivalves showed less vaccriation in zinc concentrations from various locations than from various species [62].

Zinc concentrations in mosquitofish samples from 24 Trinity River sites ranged from 7.2 to 44.7 mg/kg, with 20 of 28 samples exceeding 28 mg/kg. For comparison, we found concentrations of 27 to 34 mg/kg zinc in mosquitofish from the rural Rio Grande River at Big Bend National Park [65].

Combined Heavy Metals

Studies of toxicity of contaminants on aquatic biota have suggested a wide range of responses of organisms to mixtures of toxic chemicals. Additive effects have been documented in some bioassay studies of aquatic organisms. Antagonistic effects (where toxicity is less for a combination of toxicants than for either toxicant alone) have also been reported. Synergistic (greater than additive) effects of lead and cadmium and additive effects of lead, mercury, copper, zinc, cadmium, and mercury have also been documented for effects of metals concentrations in water on aquatic biota [29]. Mixing various combinations of mutagens has also produced both synergistic and antagonistic effects [85].

Mixtures of trace metal contaminants in sediments often result in fewer species of fish and invertebrates, as sensitive species are eliminated [95]. For example, in the Irwell River (England), sediments contaminated by high levels of lead, zinc, and copper, were thought to be responsible for the lack of fish and the paucity of invertebrates, particularly since sufficient oxygen was present [96]. Combinations of low levels of aluminum, cadmium, copper, and hardness were shown to be toxic to striped bass in the Chesapeake Bay at moderate pH levels [118].

Little is known concerning whether body burden combinations of toxic chemicals can cause greater effects on prey species and the predators consuming them than the effects of any single contaminant. Fish and wildlife from polluted areas typically carry body burdens of several different toxic chemicals. Either additive or synergistic responses would result in cumulative impacts.

Predator Alert Level: A level of 0.5 mg/kg has been suggested as a high level at which arsenic, cadmium, lead, mercury, or selenium would harm fish [20]. We totalled the concentrations of these metals to get a cumulative measure of accumulation of heavy metals. Since so little is known concerning whether body burden combinations of toxic chemicals can cause greater effects than the effects of any single contaminant, we compared the resulting summation with 0.5 mg/kg, a predator alert level we are utilizing for discussion purposes only.

Only three of 50 samples for which we sampled all five metals were below 0.5 mg/kg. These five included mosquitofish whole-body samples from sites 15, 16, and 22, all locations off the mainstem of the Trinity River. The highest seven values (3.12 to 7.66 mg/kg) were all from mosquitofish or turtles from polluted storm drains or from the mainstem of the Trinity River below Dallas. Clams tend to be good accumulators of heavy metals [95], and clam (flesh) samples had higher concentrations of combined heavy metals than mosquitofish samples at sites where both were collected. However, few or no clams were found at most of the sites which were highly impacted by pollution.

Hopefully, future research will provide more information on the effect of combined heavy metals on fish and wildlife and the predatory species eating them.

Heavy Metals and Organochlorine Pesticides

Concentrations of arsenic, cadmium, lead, mercury, chromium, and selenium were added to get a cumulative measure of toxic metals, and the results were compared to a total organochlorine pesticide measure obtained by totalling the concentrations of all chlordane isomers, DDE, DDD, dieldrin, heptachlor epoxide, and lindane.

Low concentrations of heavy metals in a given sample were not always accompanied by low concentrations of organochlorine pesticides. Only 3 of the 10 samples having the lowest concentrations of total metals were also in the 16 samples having the lowest concentrations of total organochlorine pesticides. Two of the 10 samples having the lowest concentrations of total metals were among the 7 samples having the highest concentrations of total organochlorine pesticides. Conversely, 3 of the 5 samples having the highest concentrations of total metals were among the 16 samples having the lowest concentrations of total organochlorine pesticides.

All of the nine samples ranked lowest in total metals were either fatty (lipid content greater than 5%) tissues or were from site 1, the control/reference site. All of the 16 samples ranked lowest in total organochlorine pesticides were either lean (lipid content less than 5%) tissues or were from sites upstream of downtown Fort Worth or Dallas.

Only two samples, composite samples of freshwater drum and mosquitofish, ranked very low for both total metals and total organochlorine pesticides, and both were from the control/reference site (#1).

Little is known about whether dietary combinations of organic and metal chemicals can cause greater or lesser effects on predators than the effects of any single contaminant, although synergistic effects have been documented for aqueous mixtures of several of the toxic chemicals present in the Trinity [47]. For example, body burdens of dieldrin apparently decrease a fish's ability to tolerate ammonia, a common pollutant in the Trinity River [57]. Ammonia has also been shown to work synergistically with several metals which are elevated in the Trinity River (see sections on copper and zinc).

Survival of striped bass was significantly reduced in laboratory tests by exposing them to a mixture of organic and inorganic contaminants at concentrations similar to those found on east coast spawning grounds [89]. The contaminants used in this experiment are common in the upper Trinity River (PCBs, PAHs, and several heavy metals). Doubling the concentrations of each contaminant in the mixture further increased the lethality of the mix and the bioaccumulation of PAHs [89].

Although the highest concentrations of metals and organics are often in different organs or tissues, stress of various types or stress affecting more than one organ may result in higher levels of cumulative stress on the organism. Cumulative stress of combinations of toxic contaminants can result in very rapid degradation of fish and wildlife in a river once a certain threshold (the system's assimilative capacity) is reached [48]. However, information on effects of specific combinations of contaminants versus body burdens in specific species is sparse. This topic needs more study (see recommendations section).

pH

In addition to creating a more acidic environment in which some metals are more mobile and toxic, low pH is positively correlated with increased accumulation of mercury by fish [120]. Measured levels of pH at our collecting sites ranged from 5.2 to 8.3.

Low levels of monomeric aluminum can be toxic to some fish species at pH levels below 7.3 [118]. Ten of our sites had pH levels below 7.3. Five had pH levels below 7.2. These five included Village Creek (pH 5.2, no fish present in area of serious chlorine leak), site 18 (pH 5.8, site of severe pollution due to illegal point-source discharges), and three highly impacted sites downstream of Dallas, site 12 (pH 6.8), site 10 (pH 7.0), and site 11 (pH 7.1). Water quality information collected separately by the U.S. Geological Survey confirmed that pH levels in the Trinity are generally above 7.3 but occasionally dropped to the 7.0 to 7.1 range in the late summer of 1985 in downtown Dallas [119]. Additional factors relating to pH are discussed in the sections on aluminum and fish kills.

Chemicals Below Detection Limits

Several contaminants were found to be below detection concentrations in all of our samples. Contaminants not detected included: hexachlorobenzene, *alpha*-BHC, *delta*-BHC, endosulfate, toxaphene, endrin, dacthal, endosulfan I, endosulfan II, o,p'-DDE, o,p'-DDD, o,p'-DDT, p,p'-DDT, and thallium. Except for thallium, the detection limit was 0.01 mg/kg. The detection limit for thallium was 0.3 mg/kg.

Comparison of Control and Impacted Sites

Mustang Creek, a small tributary to Benbrook Lake, was chosen as a control/reference (presumably clean) site, since it is above all known sources of urban pollution. The watershed was inspected from the air to confirm previous reports of no known pollution sources other than the cattle pastured there. The Trinity River in the vicinity of the I-20/Loop 635 bridge just south of Dallas was chosen as the comparison site because of known urban runoff and point-source discharges from Dallas and Fort Worth. Previous studies by other agencies have indicated highly elevated contaminant levels in river sediments from South Loop 12 to the I-20/Loop 635 area [71], but no comprehensive surveys of fish contamination have previously been undertaken.

Four tissues were selected as gradient monitoring indicators of body burdens of organic contaminants due to their high content of lipids (>5%) and availability at both sites. The fatty tissues selected for this purpose included: 1) whole-body samples of spiny softshell turtles (*Trionyx spiniferus*) , 2) fatty tissues dissected from red-eared slider turtles (*Trachemys scripta*), 3) whole-body samples of smallmouth buffalo fish (*Ictiobus bubalus*), and 4) whole-body samples of carp (*Cyprinus carpio*).

A Wilcoxon signed rank statistical test for paired samples was utilized to simultaneously compare ranks for all four tissue samples for all organic contaminants found to be elevated in at least one of the two sites. The differences between contaminant levels at the two sites were statistically significant. Of 37 comparison pairs where organic contaminants were detected at either site, concentrations were higher at the impacted site 36 times (97% of cases). The only exception was for heptachlor epoxide which was higher at the pasture land control/reference site than at any other location (see separate discussion on heptachlor epoxide).

Four other tissues were selected as gradient monitoring indicators of body burdens of metal contaminants due to their low content of lipids (<5%) and availability at both sites. The relatively lean tissues selected for this purpose included: 1) whole-body samples of bullhead minnows (*Pimephales vigilax*), 2) muscle tissue dissected from red-eared slider turtles (*Trachemys scripta*), 3) shell tissue dissected from red-eared slider turtles (*Trachemys scripta*), and 4) whole-body samples of mosquitofish (*Gambusia affinis*).

A Wilcoxon signed rank statistical test for paired samples showed that metals levels in these four tissues were significantly different at the two sites. Of 41 comparison pairs where concentrations of metal contaminants were detected at either site, concentrations were higher at the impacted site 38 times (93% of cases). The only exceptions were zinc (higher at the control/reference site in two of the four samples) and manganese (higher at the control/reference site in one of four samples). Small body burdens of zinc and manganese are not considered harmful to fish and wildlife, so these exceptions do not indicate a polluted condition at the control/reference site.

Pooling the organics and metals data provides an overall illustration of the degree to which contaminants were elevated at the impacted versus the control/reference site. Twelve metals and 14 organics contaminants were found in indicator tissues in at least one of the two sites. We analyzed the 77 instances in which any one of these contaminants was detected at either site in any of the indicator tissues of the six species of fish and wildlife collected. This analysis revealed that in 73 instances (95% of the comparisons) the contaminant concentrations were higher at the impacted site than at the control/reference site.

Mosquitofish Use for Gradient Monitoring

Although mosquitofish do not generally accumulate organic contaminants to levels as high as more fatty species such as softshell turtles or smallmouth buffalo fish, they do appear to serve well as comparative indicators of the elevated presence of both organic and metal contaminants at numerous sites in a polluted river. Normal mosquitofish populations are resistant to dispersal [64], and fish tend to be (relatively) sedentary during the summer months when many biological surveys are conducted [113].

Clams and other benthic organisms would be superior from the standpoint that they are even more immobile, but clams were absent from most polluted sites and other benthic organisms were highly variable. Although clams are good accumulators of many heavy metals, they are not as good for gradient monitoring of many organic contaminants.

Our data showed elevations of contaminants in mosquitofish in areas sampled where previous studies had revealed elevated levels of contaminants in sediments. For example, only one of 24 sites had highly elevated levels of nickel in mosquitofish tissues, and that particular site (number 4), a storm drain in downtown Fort Worth, had been the location of a nickel spill a few months earlier. Mosquitofish from two nearby stations did not have highly elevated levels of nickel, so the mosquitofish in the storm drain served as good markers for the location of the spill.

Mosquitofish body burdens showed a general trend towards higher concentrations downstream of Dallas/Fort Worth for most contaminants, which agrees well with 1) previous data on sediments [7,91] and 2) our intensive analysis of multi-species differences at the control and impacted sites (see previous section). Mosquitofish were also only one of two species which were uniformly low in combinations of both metals and organochlorine pesticides at the control/reference site (site 1).

Overall, we consider mosquitofish to be an acceptable choice for gradient monitoring of most of the chemicals analyzed in this study. Our initial information suggests that spiny softshell turtles may also be appropriate for gradient monitoring of organic contaminants. Mosquitofish appear to be better for gradient monitoring of metals. Mosquitofish are small and easily collected, which makes collecting and handling large numbers of them simple. Large sample sizes facilitate statistical analyses.

Upstream/Downstream Contaminant Trends

Contaminants which showed a tendency to increase from upstream to downstream in mosquitofish at multiple sites included aluminum, beryllium, cadmium, chromium, iron, mercury, nickel, dieldrin, and *cis*-chlordane.

A few metals, including zinc, manganese, chromium, arsenic, beryllium, iron, nickel, and aluminum, were as high or higher in body burdens of fatty (>5% lipid content) tissues at the control/reference site (site 1) as at the impacted site below Dallas (site 11), but these exceptions never occurred in whole-body mosquitofish samples, and occurred in only 3 of 41 instances in lean (<5% lipid content) tissues of other species. Only one of these chemicals, arsenic, was included in our combined list of heavy metals most harmful to predators.

Urban Runoff Versus Contaminants

Residential Runoff

All sites were given ranks for presence of residential runoff based on the extent to which housing areas were present in the drainage basin. Those sites where there were few if any residential areas in the drainage basin, such as our rural control/reference site, were assigned a rank of 1. Ponds in exclusively industrial areas were also assigned a rank of 1 if they did not receive runoff from residential areas. Those sites heavily influenced by residential runoff, such as creeks draining suburban areas, were assigned a rank of 3. Sites with intermediate influence of residential runoff, including sites not clearly falling into either of the other two categories, were given a rank of 2. A wide range of city maps, aerial photography, aerial observations, field inspections, city reports, land use maps, and discussions with field survey crews were utilized in assigning ranks.

Mosquitofish body burdens of iron, dieldrin, combined chlordane, all chlordane components, aluminum, and lead were significantly lower at sites having little or no presence of residential runoff than at other sites. Correlation coefficients between these variables were most significant ($P<0.02$) for oxychlordane, *trans*-nonachlor, *cis*-nonachlor, combined chlordane, and dieldrin.

Chlordane appears to be strongly associated with residential runoff. In another recent sampling program conducted by the author, no chlordane was detected in 27 samples of fish and wildlife from the Rio Grande River at Big Bend National Park, a very rural area with no residential runoff. An analysis of Trinity River data reveled that no residues of *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, or oxychlordane were detected in mosquitofish from the three sites having little or no presence of residential runoff. *Trans*-nonachlor was present at low levels (0.01 mg/kg) at two of the three sites, but its values were still significantly lower at sites having little or no residential runoff than at other sites. These results implicate residential areas as a significant source of chlordane in urban runoff (see chlordane discussions for additional detail).

Industrial Runoff

All sites were given ranks for presence of industrial runoff based on the extent to which industrial areas were present in the drainage basin. Those sites where there were few if any industrial areas in the drainage basin, such as our rural control/reference site or creeks in residential areas having no industrial development, were assigned a rank of 1. Those sites heavily influenced by industrial runoff, such as creeks or ponds draining highly industrial areas, were assigned a rank of 3. Sites with intermediate influence of industrial runoff, including sites not clearly falling into either of the other two categories, were given a rank of 2. A wide range of city maps, aerial photography, aerial observations, field inspections, city reports, and land use maps were utilized in assigning ranks.

PCBs, lead, chromium, and iron levels in mosquitofish were significantly lower at sites where there was little or no influence of industrial runoff than at other sites. Of these variables, PCBs had the most significant statistical correlation with presence of industrial runoff.

Downtown Runoff

All sites were given ranks for presence of immediate runoff from downtown Fort Worth or downtown Dallas based on the their proximity to the drainage basin. Those sites not downstream of downtown areas, such as our rural control/reference site or creeks in residential areas not receiving runoff from downtown areas, were assigned a rank of 1. Those sites downstream of the city centers of Dallas or Fort Worth, and no farther than four miles from downtown areas, were assigned a rank of 3. Sites with intermediate influence of downtown runoff, including sites not clearly falling into either of the other two categories, were given a rank of 2.

Lead and PCBs were the only contaminants significantly lower in mosquitofish from sites having little or no runoff from downtown areas than those from other sites. The highest levels of PCBs found in any species at any site were found in softshell turtles in a canal fed by a storm drain in downtown Fort Worth (see PCB section). However, an analysis of all mosquitofish samples revealed that lead had a closer ($P<0.008$) statistical correlation with presence of downtown runoff than did PCBs.

Sites 18, 19, 23, and 25 were storm drains and creeks in downtown Fort Worth. This group of sites had significantly higher levels of lead in mosquitofish than the sites upstream of Fort Worth. The highest single values for lead (3.7 mg/kg), nickel (12 mg/kg), and chromium (9.7 mg/kg) in mosquitofish were also found within this group.

Raw Sewage Runoff

Raw sewage discharges and overflows are common in Fort Worth and Dallas when rainfall infiltrates into leaky sewage lines and overloads the collection and treatment systems [91]. Manholes then overflow, and bypass systems release raw sewage into storm drains. Other common sources of raw sewage discharges include undersized pipes, large pipes connected to smaller pipes as sewage lines pass across city borders, septic haulers who dump along rural roads, and illegal sewage line taps as a source of waste grease---which plugs sewer lines (Ross Muir, Tarrant County Health Department, personal communication).

At the time of our 1985 collections, there were also numerous constant sources of illegal raw sewage discharges in Fort Worth due to leaky pipes and improper connections to storm drains. Although raw sewage discharges are actually illegal point-sources (single pipe or single point discharges), the effects of such discharges are usually noticed in urban creeks and storm drains and have typically been classified as urban runoff.

All sites were given ranks for presence of raw sewage runoff based on the extent to which raw sewage discharges were known to be present in the drainage basin during the rainfall event which occurred just before our collections began. This rainfall event was associated with the first large fish kill of the summer of 1985 [7]. During our collections, we observed some large raw sewage discharges in both Fort Worth and Dallas. We also obtained additional information on the location of areas of frequent discharge of raw sewage from officials of the cities and from the Texas Water Commission. Coprostanol, a chemical used as an indicator of raw or poorly treated sewage, has recently been reported to be about as high in sediments from our site number 9 as it is in sewage sludge from the ocean dumping region used by New York City; this is thought to be indicative of a major impact of inadequately treated sewage in the Trinity River just below Dallas [91].

Those sites where there were few if any areas with raw sewage discharge in the drainage basin, such as our rural control/reference site, were assigned a rank of 1. Those sites where there was a significant presence of raw sewage at the time of our collections were assigned the rank of 3. Sites with intermediate influence of raw sewage runoff, including sites not clearly falling into either of the other two categories, were given a rank of 2. Our personal observations, city and state records, and consultations with city officials, were utilized in assigning ranks.

In an analysis of mainstem sites, mosquitofish levels of mercury were significantly lower at sites where there was little or no influence of raw sewage than those from other sites. In analyses of both mainstem sites and all mosquitofish samples, lead had an especially close ($P<0.02$) positive, statistical correlation with the presence of raw sewage.

Since we expressed our concern to them in 1985, the City of Fort Worth Health Department has developed an excellent "storm drain team" and has shown leadership in tracking down and eliminating illegal discharges of raw sewage.

Point Source Discharges Versus Contaminant Levels

Chlorinated, Treated Sewage

Due to the presence of several high-flow sewage treatment plants, the Trinity River from eastern Fort Worth to well below Dallas is truly "effluent-dominated." When the river is very low, up to 90% of the flow of the river originates as treated, chlorinated sewage.

All sites were given ranks for presence of chlorinated, treated sewage based on the extent to which effluents from sewage treatment plants influenced water quality at the site. Those sites where there were no sources of chlorinated, treated sewage, such as our rural control/reference site or creeks above the influence of sewage treatment plants, were assigned a rank of 1. Those sites heavily influenced by chlorinated, treated sewage, such as those just downstream from very large sewage treatment plants, were assigned a rank of 3. Sites with intermediate influence of chlorinated, treated sewage, including sites not clearly falling into either of the other two categories, were given a rank of 2. The proximity of upstream sewage treatment plants and dilution factors were the main factors utilized in assigning ranks.

Levels of aluminum in mosquitofish were significantly lower at mainstem sites where there was little or no influence of treated sewage than at other sites. In analyses of both mainstem and all mosquitofish sites, aluminum also showed a strong correlation with presence of treated sewage, confirming the river mile and location group trends documented in the aluminum section of this report.

Mercury levels in mosquitofish also had positive statistical correlations with presence of treated, chlorinated, sewage in analyses of mainstem-only sites and all sites.

Impacts on Populations of Small Fish

Upstream Versus Downstream

Simple measures of biological integrity (such as the number of species present) have been found to useful in assessing aquatic life impacts of various stress factors [50,110,111,112]. More complex derived indices (such as species diversity) are often less useful than simple measures in demonstrating the impact of toxic chemicals, since the derived variables often show little variation between control and impacted sites and since their sampling distribution is unknown [110,112].

While we were conducting equal-effort seining for mosquitofish, we identified, counted, and categorized all species of small fish collected. This data was used to generate two fish population metrics: percentage of species considered pollution-tolerant (% POLTOL), and percentage reduction from the number (13) of species collected at the control/reference site (% REDUC13).

Species collected which we consider to be pollution-tolerant included mosquitofish, *Gambusia affinis*; bullhead minnows, *Pimephales vigilax*; and black bullhead catfish, *Ictalurus melas*. Although its laboratory resistance to chemicals is less consistent, the red shiner, *Notropis lutrensis*, was also included as a pollution-tolerant species, because our experience has included finding it in a variety of polluted habitats. Other investigators have also designated it as pollution-tolerant [6]. Classifying the green sunfish, *Lepomis cyanellus*, was more difficult. Although it is tolerant of intermittent streams in extreme headwater situations and tolerates turbid and organically enriched conditions, we could not confirm *Lepomis cyanellus* as truly pollution-tolerant. We excluded it, since our experience suggests that it is often absent from areas of chemical pollution and at least one other study [52] has shown it to be no more tolerant of petroleum effluents than other species of sunfish.

The "% REDUC13" population metric is simply the number of species collected expressed as a percentage reduction from the number (13) of species collected at the control/reference site. We would normally expect to find a higher number of fish species at higher-order, downstream sites where the river has more water volume than at upstream sites [50]. However, Trinity River sites downstream of Arlington became progressively harder to seine due to deeper water, steeper banks, softer sediments, and deeper deposits of fine sediments. Our ability to collect the increased number of fish species expected at downstream sites was cancelled out at least somewhat by the more difficult collecting conditions. Therefore, the "% REDUC 13" metric is considered useful as an approximate measure of impact for preliminary survey purposes.

A third fish population metric, percentage reduction of species expected (% REDUCTION), was developed for comparison to the other two metrics. Best professional judgement of the author, in consultation with several ichthyologists familiar with the Trinity River, was utilized to estimate which species of small fish should have been collected at each site, if the water and bottom sediments were clean but flow regime and stream morphology were the same as they are now. An actual list of the species which would have been expected at each site was generated and collection efficiencies at each site were estimated and factored into all calculations.

After completing this procedure, the decision was made to eliminate the % REDUCTION metric for purposes of population impact analysis, since it is more qualitative than the other two procedures. Little information was lost by dropping the % REDUCTION metric, since it tracks so well with the other two metrics. However, in response to requests by other investigators, we have included it in Figure 17 for comparison purposes. Figure 17 summarizes all three fish population metrics from Mustang Creek to the Highway 79 area.

Figure 17 shows an upstream to downstream progression from little or no impact on fish populations at Mustang Creek to some impact in downtown Fort Worth, greater impact just downstream of Fort Worth, and severe impacts from the first large sewage treatment plant (Fort Worth's Village Creek Plant) to an area well south of Dallas. Even though our data reflected observations from only one point in time, the temporal proximity of our collections to both 1985 fish kills gives the observations added significance.

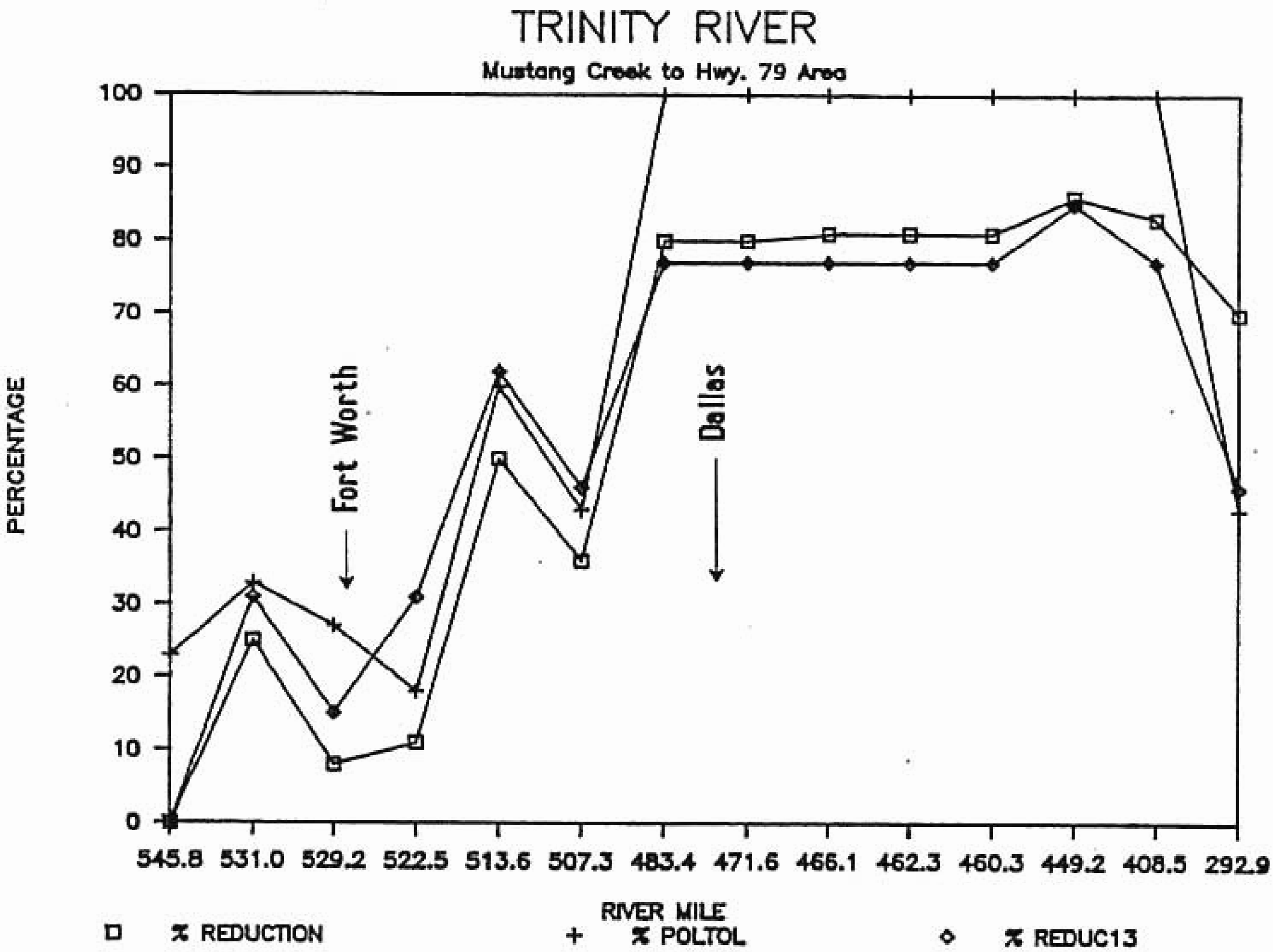


Fig. 17. Fish population impact metrics versus river mile.

Runoff Type Versus Fish Populations

Spearman rank correlation coefficients showed neither positive nor negative correlations between residential runoff, industrial runoff, or downtown runoff and either of the fish population metrics, percentage pollution tolerant or percentage of species collected at the control/reference site. This finding reflected steady state conditions at the time of our collections. Scatter plots and statistical analyses of location groups showed some association between industrial runoff and the fish population metrics, but the association was not a straight line correlation and the correlation coefficients were not significant. However, storm induced surges of polluted water having high instantaneous chemical oxygen demand (COD) have been shown to be a cause of sudden drops of dissolved oxygen in the Trinity [49].

One of the causes of the excess COD could be a build up of industrial chemicals and non-toxic organic wastes from numerous urban sources. Thus, some of the episodic stress on fish in the river may be attributable to industrial or downtown runoff.

Raw Sewage Influence

Spearman rank correlation coefficients and simple plots showed a positive correlation between raw sewage runoff and the average of the two fish population impact metrics, percentage pollution tolerant and percentage of species collected at the control/reference site. Increasing influence of raw sewage showed a general trend towards increasing impact on fish populations.

Influence of Chlorinated, Treated Sewage

Spearman rank correlation coefficients showed a positive correlation between chlorinated, treated sewage and both of the fish population metrics, percentage pollution-tolerant and percentage of species collected at the control/reference site. Sites having little or no presence of chlorinated, treated sewage had significantly lower values for the average of the two fish population impact metrics, reflecting less impact on the fish populations (Fig. 18).

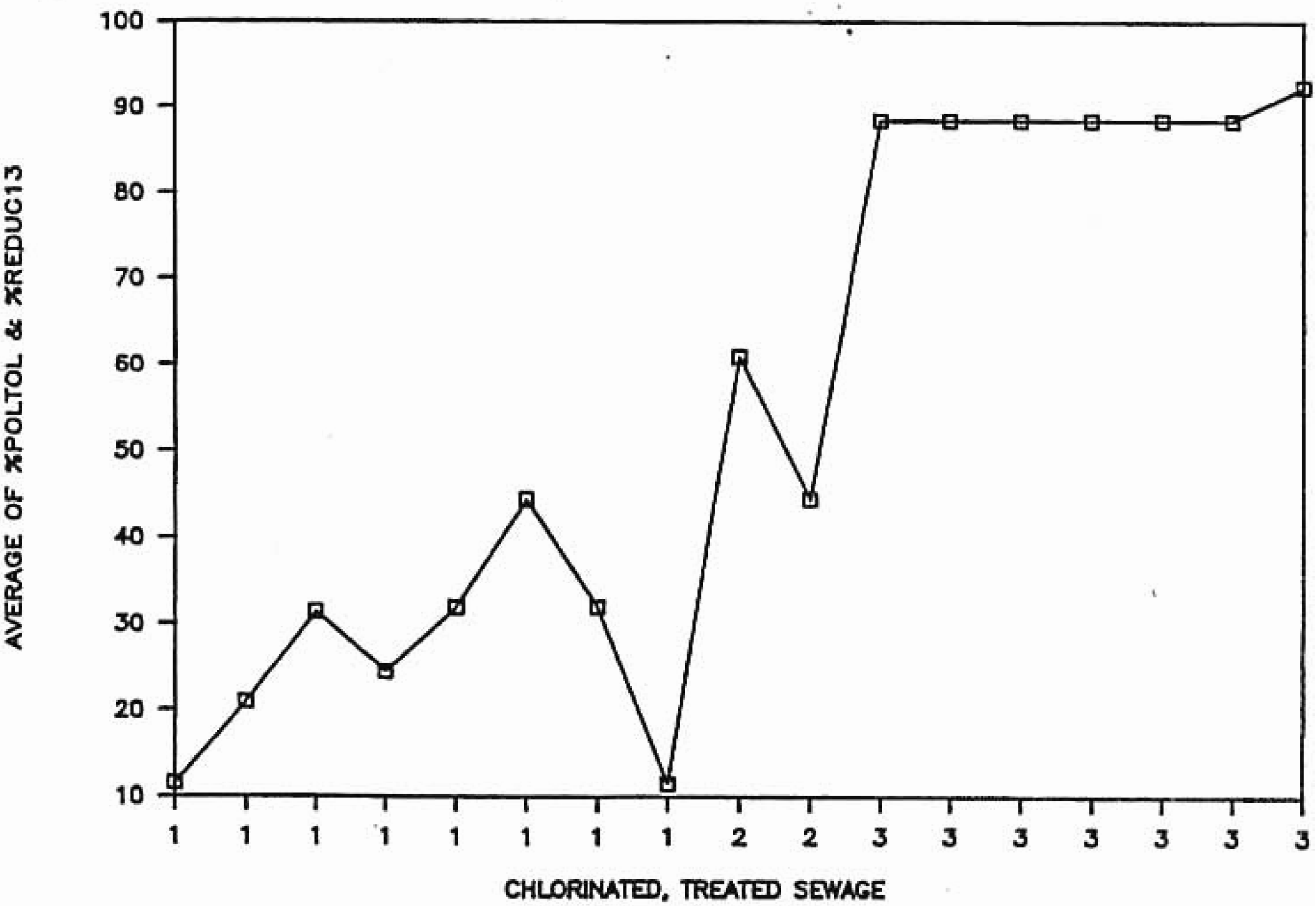


Fig. 18. Average of fish population metrics, percentage pollution tolerant (%POLTOL) and percentage reduction from the number of species collected at the control/reference site (%REDUC13), compared to ranks (low=1, medium=2, high=3) for the presence of chlorinated, treated sewage.

Low Oxygen Levels

Since low oxygen levels have been implicated [7] as an important factor in Trinity River fish kills, our data were analyzed for correlation between oxygen levels observed at our collecting sites versus fish population impact metrics.

Those sites where there was no depression of dissolved oxygen from normal levels, such as our rural control/reference site, were assigned a rank of 1. Those sites where dissolved oxygen levels at the time of our collections were depressed below 5.0 ppm were assigned the rank of 3. Sites with intermediate influence of low dissolved oxygen levels, including sites not clearly falling into either of the other two categories, were given a rank of 2.

As shown in Fig. 19, presence of low dissolved oxygen correlated less well with degree of impact to fish populations than did the presence of chlorinated, treated sewage (Fig. 18). We collected carp showing no apparent signs of distress at site 11 when the dissolved oxygen concentration was as low as 0.7 parts per million.

However, low oxygen undoubtedly plays a role in fish kills, including rapid oxygen depletion resulting from chemical oxygen demand (COD) upstream following rise events and biological oxygen demand (BOD) exerting additional stress downstream.

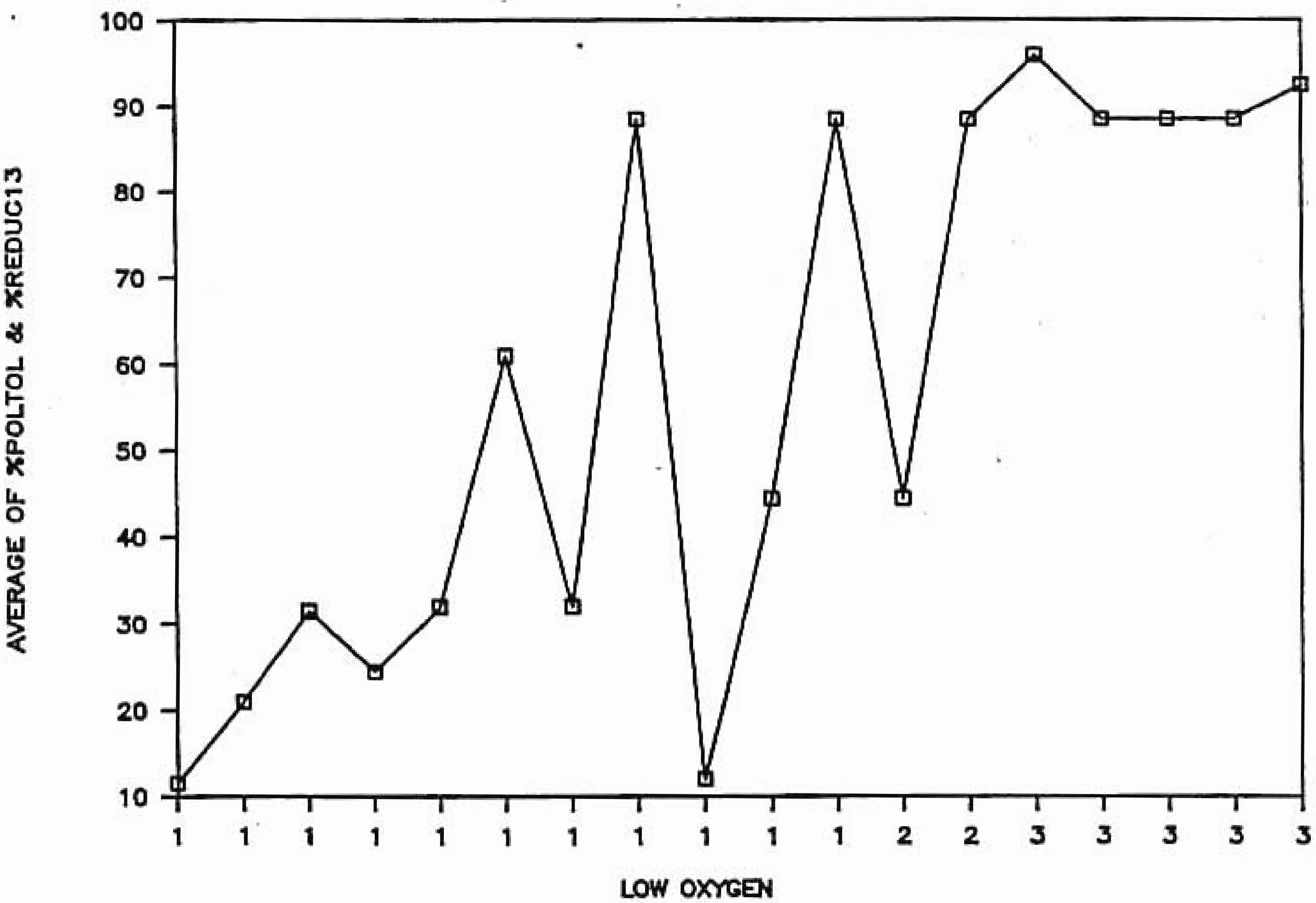


Fig. 19. Average of two fish population impact metrics, percentage pollution tolerant (%POLTOL) and percentage reduction from the number of species collected at the control/reference site (%REDUC13), compared to ranks (low=1, medium=2, high=3) for the presence of low levels of dissolved oxygen.

Principal Components

Fig. 20 displays two linear combinations of impact variables, PC#1 and PC#2, correlated with average impact on small fish populations.

PC#2 was calculated for each site by giving equal weighting to previously assigned 1, 2, or 3 ranks for all five runoff variables: residential runoff; industrial runoff; presence of chlorinated, treated sewage; presence of low oxygen levels; and presence of raw sewage. Each of the runoff variables had a potential maximum of 3 points at each site, or a potential maximum of 15 (3 x 5) for all 5 variables.

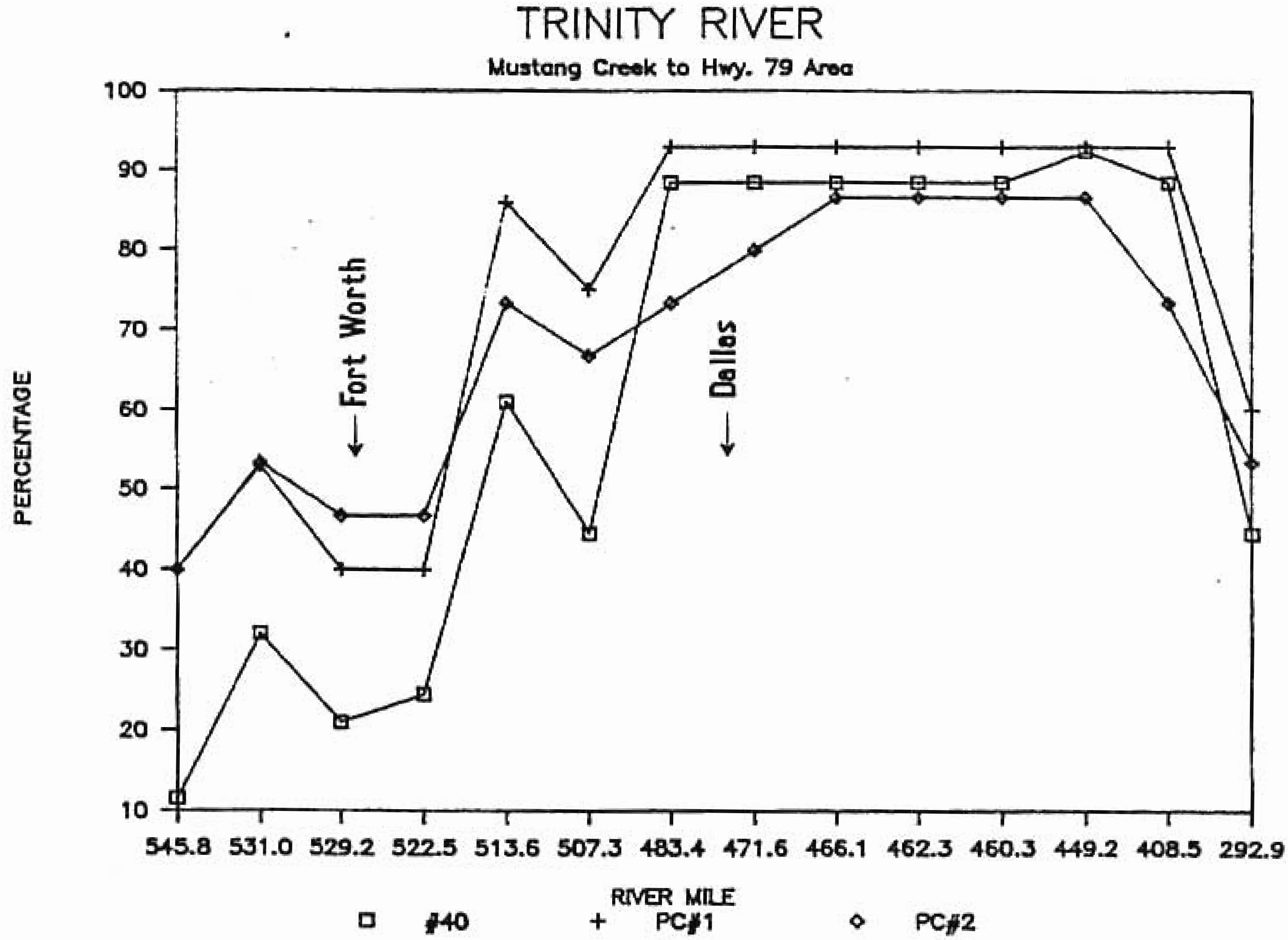


Fig. 20. River mile comparison of variable #40 [average of two fish population impact metrics, percentage pollution tolerant (%POLTOL) and percentage reduction from the number of species collected at the control/reference site (%REDUC13)], compared to PC#1 and PC#2 (defined in the text).

As shown in Fig. 20, there was a fairly good correlation between additive runoff impacts (PC#2) and average impact on populations of small fish. This demonstrates there may be some validity to the theory that cumulative population impacts from different types of runoff can gradually increase as a river flows downstream.

A cumulative impact variable (PC#1) which correlated even more closely with overall impacts on fish populations was created by the following procedure: (1) eliminating residential runoff, downtown runoff, and the presence of low dissolved oxygen; (2) giving double weighting to presence of chlorinated, treated sewage and to a chlorine- adjusted raw sewage variable; and (3) giving single weighting to presence of industrial runoff (which had a non-linear correlation to fish population variables). The resulting variable, PC#1, is plotted in comparison to PC#2 and average impact to fish populations in Fig. 20.

At the time of our collections, sites 5 and 6 (river miles 513.6 and 507.3) were downstream of notable amounts of raw sewage and industrial runoff (Ross Muir, Tarrant County Health Department, personal communication). These are potential factors in the slight depression of fish populations observed at those sites. In calculating PC#1, presence of raw sewage was given a different ranking scale which correlates better with fish population impacts. Those sites with some well diluted raw sewage but no known presence of chlorine were assigned the rank of 1. Those sites with little or no raw sewage were assigned a rank of 2. Those sites with at least some raw sewage in the presence of chlorine were assigned the rank of 3. All other runoff variables retained the original ranks (1=little or no presence, 2=intermediate presence, 3=significant presence) assigned in previous sections of this report.

The apparent importance of chlorine to fish populations is underscored by the fact that the two (double-weighted) variables which had the closest correlations with fish population impacts were related to the presence of chlorine. An additional indication of the probable importance of chlorine was obtained through an analysis of data from all sites, which revealed that treated, chlorinated sewage was more highly correlated (P=.0007) with average impact on fish populations than was presence of low levels of dissolved oxygen or the presence of any type of urban runoff.

Chlorine Impacts

Since our initial findings suggested chlorine appeared to be playing an important role in fish distribution, we enlisted the aid of an undergraduate student at Texas Christian University, Kirk Dean, to run some instream in situ toxicity tests downstream from chlorinated discharges. This work was conducted as part of the U.S. Fish and Wildlife Service volunteer program. Kirk's findings (the entire report is presented in Appendix 2) include evidence that 1) chlorination of sewage treatment plant effluents causes significant mortality of some fish species as far as five miles downstream, 2) toxic substances are being discharged in illegal (toxic) amounts at the plants tested, and 3) sometimes there is no legally required zone of passage allowing fish to move up or down river without passing through a zone of toxicity. The limited data in Kirk's report are insufficient to expand such generalizations to all Trinity River sewage treatment plants at all times. However, these findings do suggest the impact of chlorine on Trinity River fish may be substantial. Kirk's findings are consistent with known toxic effects of chlorine in the amounts it is being discharged [104,105]. The toxicity is probably greatest at low flow conditions or when the sewage plants are discharging more than the usual amount of chlorine.

During our collections, we discovered a chlorine leak from Fort Worth's Village Creek Sewage Treatment Plant which had totally obliterated all aquatic life in Village Creek just upstream of its confluence with the Trinity.

Many aquatic species are very sensitive to chlorine at very low levels [104,105]. Low levels of chlorine may produce changes in community structure [108]. Taxa are eliminated from chlorine stressed systems at levels of stress below levels which would result in net biomass loss [105]. This pattern of lower species diversity accompanied by high biomass of a few resistant species is the same pattern we observed in fish populations downstream of the big chlorine sources (sewage treatment plants) on the Trinity River.

A previous study on the Trinity documented that chlorine was resulting in a drastic reduction of snails and other invertebrates just downstream of a sewage treatment plant [54]. In another study, protozoan species were reduced by 25% at the very low chlorine doses of 6.1 ug/l (parts per billion) and algal biomass was adversely affected at 2 ug/l [105]. Our data, in addition to other data in the scientific literature [73,104,105,108], lead us to believe that chlorine is probably a significant factor helping to exclude some species of fish from sections of the river downstream from the large sewage treatment plants.

Investigators in Illinois found that fish populations downstream of a sewage plant with secondary treatment and chlorination were severely impacted, while fish populations downstream of a sewage plant with secondary treatment but no chlorination were enhanced [50].

Another more recent study of three rivers in Illinois provided similar, more detailed results [73]. These results appear to be very pertinent to the Trinity River. As we have documented for the Trinity, fish populations below the three large sewage plants in Illinois were highly impacted compared to fish populations upstream of the chlorinated sewage plant effluent [73]. Stopping chlorination of secondary treatment effluents at these sewage plants resulted in complete recovery of fish populations downstream [73]. The benefit to fish and wildlife populations downstream was greater from stopping chlorination of secondary treatment effluents than the benefit observed from providing (expensive) tertiary treatment of the effluent in addition to stopping chlorination [73].

The same study [73] also concluded that areas farther downstream, where only a weak effluent was present, nevertheless showed clear improvements following cessation of chlorination. Our study of the Trinity River was conducted at a time when it was being influenced by chlorine from numerous large sewage plants and at least one chlorine leak. Our observation was that fish populations did not fully recover even 200 miles downstream of the sewage plants. However, the presence of other toxic chemicals and stress factors makes it impossible to single out chlorine as the only cause of negative impacts on fish populations. Ongoing studies by other agencies should give us a better idea of how these impacts vary over longer periods of time and in various flow regimes.

Chloramines are oxidizing agent chemicals which are used to disinfect Dallas/Fort Worth drinking water. Like chlorine, chloramines are also highly toxic to fish. Chloramines are a combination of ammonia, which is already elevated in the Trinity River, with chlorine. As a nitrogen source, chloramines may also indirectly help produce potentially harmful nitrogen-containing compounds such as organic amines and chloronitriles [101]. The presence of organonitrogen compounds reduces the effectiveness of chlorine as a disinfectant [101].

Fort Worth Storm Drain Team investigators have discovered numerous places where chloramine tainted water is entering the river via leaky distribution pipes (Gene Rattan, City of Fort Worth, personal communication). Chloramines are also formed in the chlorination of sewage effluents due to the presence of ammonia [73].

In addition to chloramines, other chemicals which are harmful (toxic, carcinogenic, or mutagenic) to fish and wildlife are formed by the chlorination of effluents which contain oxidizable organic impurities [106,107]. The degree of chlorination of many organic compounds is related to the degree of toxicity or other hazards it poses for living organisms. For example, mixtures of PCBs with a high degree of chlorination produce liver cancer in rats, while PCB mixtures with less chlorination yield much lower cancer rates [114].

Most of the attention focused on chlorination by-products has been concerned with potential production of harmful chemicals as a result of disinfection of drinking water (101). However, the greater the concentrations of organic impurities, the greater the hazard for creating harmful chemicals as by-products (101). Therefore, the problem of creation of harmful by-products should be more serious in the chlorination of treated sewage than in the chlorination of filtered drinking water, and greater yet in the chlorination of raw or poorly treated sewage.

Chlorine and other chemicals used to disinfect treated sewage act as strong oxidizing agents of phenols, aromatic amines, amino acids, and pesticides [101]. Strong oxidants can transform pesticides like parathion and malathion to initially more toxic intermediates and heptachlor to heptachlor epoxide [101]. Vigorous oxidation of humic materials sometimes liberates entrapped or complexed toxic pesticides (such as lindane) or heavy metals [101]. Other harmful chemicals which have been produced as by-products of the chlorination of organic-enriched effluents have included: ring-chlorinated aromatics, cyano-substituted compounds (nitriles), chlorinated aliphatichydrocarbons, and chlorinated aliphatic aldehydes [101]. As mentioned above, the presence of significant amounts of ammonia in sewage discharges plays a role in the production of chloramines [73] and probably other nitrogen-containing, chlorinated, toxic compounds.

Carcinogenic and teratogenic trihalomethanes (THMs) are also formed by the chlorination of effluents containing organic matter [8,102]. Except for chloroform, the THMs formed by chlorination of drinking water are also mutagenic [102].

The highest concentrations of THMs in drinking water are reported from localities which chlorinate surface water rather than ground water [102]. Chloroform is the most commonly occurring THM in disinfected drinking water and treated sewage [102]. Chloroform is also formed in other effluents containing aliphatics, humic matter, and other organic precursors [8,101,102].

Very low (3-180 ug/kg) concentrations of chloroform have been reported in tissues of edible, marine fish and wildlife [102]. These low levels are nevertheless considered to be high enough to cause a small increase in cancer risk to the humans which consume them [102]. These human risk estimates were based partly on animal tests, so fish and wildlife may also be at risk from consuming food or water contaminated by chloroform. Chloroform has been found to induce thyroid, liver, and kidney cancer in small mammals and was estimated to have accounted for 70% of the risk associated with the highly publicized Love Canal leachate [85]. Several epidemiology studies have shown strong correlations between chloroform concentrations in drinking water and cancer rates [102].

The potential for formation of THMs may be relevant to the Trinity River due to the considerable amount of chlorine entering the river in effluents of large sewage treatment plants. Very low levels of chloroform are acutely toxic to aquatic life [102]. The toxic effects of chloroform are increased by pretreatment with DDT and a few other chemicals [102].

There is no appreciable decomposition of chloroform at ambient temperatures in water, even in sunlight [102].

However, like chlorine, chloroform is subject to potential loss to the atmosphere, so the most pronounced toxic effects in a river may occur at locations close to continuous sources of chlorinated effluent.

At present, we do not know what proportion of the adverse effects of chlorine on fish and wildlife in the Trinity River are the result of free chlorine versus chlorination by-products such as chloramines, trihalomethanes (such as chloroform), lindane, amines, organic cyanides (nitriles), or other potential toxic chemicals which are formed or liberated as the result of chlorination of effluents containing organic matter. However, limiting or stopping chlorination of sewage effluents should reduce the concentrations of all of these chemicals downstream of the effluents.

Fish Kills

Although this study was not designed to answer the question of what is causing the recurrent fish kills in the Trinity River below Dallas, some of our observations are relevant to the issue. In response to requests from other agencies and individuals, we are providing these observations and a discussion of their possible meaning in light of other information that is available to us.

On July 25, 1985, our field crew observed 18, large, dead smallmouth buffalo fish and channel catfish floating down the river at South Loop 12 (site 9), one day before the second large fish kill of 1985 was reported downstream. Dissolved oxygen readings were not low in the immediate area. The fish were estimated to have been dead for about a day. Given the flow velocity of the river, their approximate location at the time of their death would have been the area near Village Creek Sewage Treatment Plant's discharge, where oxygen levels were not depressed a day earlier [56]. Chlorine levels, however, would still have been elevated at that time due to a chlorine leak from the sewage plant into the river.

Another Department of Interior field crew reported a similar fish kill (a limited number of fish at site 9) later that fall, also when oxygen levels were not depressed (William Herb, U.S. Geological Survey, personal communication). Neither this fish kill nor the one we observed was reported to any agency by citizens, which is not surprising since few citizens observe the river closely between sites 9 and 11.

When fish are dying and oxygen is not depressed, toxic chemicals are logically suspect, especially in an area having some of the highest reported freshwater levels of toxic chemicals anywhere in the State. We agree with Texas Parks and Wildlife Department's observation, that in the Dallas area, dissolved oxygen sags are usually not of long enough duration to kill fish [56].

It has been documented that fish can make contaminants in sediments more bioavailable by moving around and disturbing the sediments [95]. It has also been shown that other types of disturbances as well as uptake by algae can help mobilize contaminants which were previously bound to sediments [95].

Our data revealed that some contaminants are present in Trinity River fish and wildlife in amounts which may be adversely impacting them and the predatory species consuming them. This suggests that these contaminants are not always bound to the sediments to the extent that they are unavailable to fish and wildlife.

We agree with the excellent analyses [7,56,91] by the Texas Water Commission and the Texas Parks and Wildlife Department, that low dissolved oxygen is playing a primary role in most of the large Trinity River fish kills. However, it has not been proved that oxygen stress alone has been the cause of all of the fish kills. The following facts are relevant to the fish kills: (1) we have observed minor fish kills where the oxygen was high; (2) fish kills do not happen every time the oxygen gets low; (3) few riverine fish kills happen elsewhere in the U.S. due solely to oxygen depletion from legal discharges of treated sewage; (4) there are many toxic chemicals present in the Trinity; (5) preliminary toxicity testing of Trinity River sediments by the Environmental Protection Agency has revealed some toxicity [91], and (6) we have documented that the river water in some locations is occasionally acutely toxic to fish (see Appendix 2).

The presence of high levels of toxic chemicals in sediments as documented in previous studies [7, 71,91] leaves open the possibility that toxics other than chlorine could suddenly be released from sediments during turbulent conditions accompanying a sudden, heavy rain. Nothing in our findings contradicts such a possibility, and the City of Fort Worth's observations of numerous, often short-duration, illegal discharges is consistent with our view that the river suffers from recurrent pulses of highly polluted water from a great variety of sources. Accumulations of heavy metals and organics in deep pools of the Ohio river were resuspended during heavy flows and were implicated in fish kills in 1962-1964 [54]. It is very difficult to accurately predict the potential toxicity of sediments containing a mixture of toxic substances, and bioavailability of contaminants such as PCBs is related to the type of sediment involved [88,95].

Sediments having finer grain sizes tend to allow a greater release of toxins than sandy sediments [95]. Such sediments are common in the fish kill zones downstream of Dallas.

Like fish kills, pulses of highly polluted water are episodic events. These pulses of highly polluted water could result from chemical spills, sudden heavy rains washing accumulated contaminants out of storm drains, re-suspension of contaminated bottom sediments by the turbulent water accompanying heavy rains, intermittent discharges of industrial waste, poorly treated sewage resulting from an upset at a sewage treatment plant, and any number of other causes. With almost four million people living near the upper Trinity River, there is probably a raw sewage leak or chemical spill going into some part of the watershed at virtually all times. Many small and seemingly insignificant sources can result in significant cumulative impacts on a small river such as the Trinity.

When large sewage treatment plants have become overloaded due to infiltration effects of a sudden, heavy rain, they have typically discharged poorer quality effluent with a higher concentration of chlorine. Not only do discharges of high levels of chlorine have potential for direct toxic effects on fish and wildlife in the river, but chlorination of raw or poorly treated sewage produces far more mutagenic chemical compounds than does chlorination of well treated sewage [51,101]. The very strong oxidizing agents used for sewage disinfection can also sometimes liberate toxic materials which were previously entrapped in humic materials (see chlorine impact section). Addition of strong oxidizing disinfectants may also impact oxygen levels in the river, since strong oxidation of humic/fulvic materials can greatly increase their biological oxygen demand [101].

Previous reports have suggested that chlorine from Trinity River sewage treatment plants may be killing invertebrates that pass through a sewage plant or are washed downstream through the toxic zone where the sewage plant effluent is mixing with river water [54]. Other reports have documented adverse impacts on algae at levels as low as 2 ug/l [105]. Phytoplankton coming in contact with the much higher levels of chlorine in sewage effluents may be killed. The problem would be most serious when chlorination levels are higher than usual.

Some algae are effective at bioconcentrating heavy metal and organic contaminants from sediments [95]. These contaminants are released to downstream sediments when the algae die and sink to the bottom [55,95]. Dead algae, invertebrates, and other aquatic organisms killed by the chlorine or other toxic chemicals in Dallas/Fort Worth are transported by currents and an increased demand for oxygen occurs farther downstream as bacteria degrade them. Thus the death of these organisms upstream may be indirectly involved in moving both toxic chemicals and oxygen demand into the downstream areas where fish kills occur.

Another source of toxic chemicals and excess oxygen demand is raw sewage discharged directly to the river. Sources of raw sewage and sewage sludge in the Trinity River include catastrophic discharges resulting from line breaks [91].

Raw sewage discharges from any source result in increased amounts of ammonia in the river. Treated sewage has also been a source of considerable amounts of ammonia, although the concentrations are lower than for raw sewage. We have already discussed the role of ammonia as a co-factor in producing toxic compounds as by-products of the chlorination process (see section on chlorine impacts).

Un-ionized ammonia has also occurred in amounts toxic to fish in the Trinity River below large sewage treatment plants and downstream of Dallas. Ammonia oxidizes to nitrites and nitrates in the river. Nitrite toxicity reduces the tolerance of fish to low oxygen [116]. Toxic effects of nitrites which have been observed in fish include: 1) oxidation of hemoglobin to methemoglobin, a form incapable of binding oxygen, and 2) reduced swimming performance [116]. Elevated levels of nitrites have been observed during rise events on the Trinity [91].

Ammonia toxicity is synergistically increased by elevated levels of copper and zinc [47], and both copper and zinc are elevated in the Trinity. Body burdens of dieldrin decrease a fish's ability to tolerate ammonia [57], and both of these pollutants are elevated in the Trinity River.

Episodic pulses of contaminated water would have their maximum impact during low flow conditions in the hottest months of the year, when fish are already in stress from loss of habitat due to low water, higher contaminant concentrations from lack of dilution water, low pH, a surplus of heat, ammonia levels at or near toxic levels, a presence of chlorine and nitrites, low oxygen levels, and considerable body burdens of complex mixtures of contaminants. Below Dallas, these stresses tend to be more continuous than episodic during mid to late summer.

Additional episodic effects such as pulses of water with even higher concentrations of ammonia, nitrites, chlorine, chemical oxygen demand, biological oxygen demand, spills or dumps of various acidic or otherwise toxic chemicals, release of toxic chemicals from re-suspension of bottom sediments during flow increases, or any combination of the above, would contribute significant additional stress. It is possible that we have not yet identified all of the toxic chemicals which may play a role in some of the fish kills (see discussion of the impacts of non-routine contaminants in the recommendations section).

Most Trinity River fish kills have occurred when water temperatures are elevated, following an extended period of low flows [91]. Zinc, a common urban contaminant which is elevated in the upper Trinity, is more acutely toxic to fish at higher temperatures than at lower temperatures [98]. Aluminum or other heavy metal toxicity in the presence of low pH is another of the many potential cofactors which may be playing a role in some of the fish kills (see discussions of aluminum and pH).

In recent years, progress has been made at large sewage treatment plants in the Dallas/Fort Worth area in improving the quality of effluents and in decreasing large discharges of raw sewage [91]. Less region-wide progress is evident in identifying the extent or reducing the amount of potential pollution from storm drains and from illegal and older municipal landfills. However, landfills and storm drains are only two of the many potential sources of toxic chemicals and high oxygen demand in the Trinity River. Neither this study nor any of the other studies to date were designed to be exhaustive surveys of all potential sources or hazardous chemicals likely to occur in the river. Studies underway (see detailed discussions in the next section) will hopefully offer additional insight. However, conditions in the river have now changed, so we may never know the extent to which toxic chemicals may have been co-factors during the 1984-1985 kills.

Flash floods in urban areas can greatly increase pollutant loads from other sources of urban runoff and result in pulses of high chemical oxygen demand [49,91]. In riverine habitats, cumulative stress of combinations of toxic contaminants can suddenly result in very rapid degradation once a certain threshold (the system's assimilative capacity) is reached [48].

RECOMMENDATIONS

Definitive Studies:

Since this study was an initial (exploratory) survey, we recommend that several more definitive follow-up studies be conducted to further investigate our initial findings. Just as our initial findings prompted us to further investigate the impacts of chlorine by conducting additional field tests (see Appendix 2), many of the other initial findings in this report require additional study for more definitive confirmation. For example, the many correlations between runoff type and elevated contaminant levels or population impacts could be more definitively confirmed through repetitive sampling at more sites over a longer period of time.

We have provided our findings to several other governmental and academic groups which are now conducting or planning additional studies on the Trinity River. We are hopeful that the results of these studies will include additional insight relating to some of the issues we have raised. Following completion of these studies, the Fish and Wildlife Service will reassess future study needs.

Development of Rapid Bioassessment Methods:

Reliance on chemical-specific criteria and effluent bioassays is insufficient for optimum protection of fish and wildlife resources [113]. In keeping with the goals of the Water Quality Act of 1987, the Environmental Protection Agency is encouraging states to develop 1) biocriteria for inclusion in state water quality standards and 2) instream bioassessment methods for measuring compliance with the biocriteria [113]. As of December, 1988, ten states had began developing biosurvey methods and/or biocriteria [113].

Although instream biosurveys are the best way to assess attainment of designated aquatic life uses, the cost and complexity of many bioassessment methods has prevented their widespread acceptance. The availability of simplified, economical methods would reduce the cost and complexity of instream studies and facilitate field surveys at a greater number of sites. The availability of economical instream bioassessment methods should also indirectly benefit fish and wildlife by making it practical to develop biocriteria for inclusion in Texas water quality standards.

Biocriteria and standardized bioassessment methods would be useful for the following applications: 1) use attainability analyses, 2) identifying areas where site-specific criteria (both biological and chemical) are needed, 3) characterizing the value of high quality waters as required by anti-degradation standards, 4) determining the need for additional controls, 5) identifying previously unknown sources of pollution, and 6) measuring attainment of the fishable/swimmable goals in the Water Quality Act of 1987 [113]. Rapid bioassessment methods would also be useful to natural resource agencies in assessing potential impacts of the numerous smaller development projects for which there is insufficient time to conduct complicated assessments.

Two simplified, rapid assessment methods were used in this report: 1) fish population impact assessments using measurements of the number of species and the percentage of pollution-tolerant species, and 2) minnow-bucket bioassays (see appendix 2). Since the University of North Texas is now conducting much more complex instream monitoring of many of the sites we utilized, it will be instructive to compare their final results to ours. This comparison will provide a rough estimate of how valuable our simplified assessment methods were in predicting the results of their more complex studies. The reasons this comparison will provide an approximate rather than a definitive estimate of the usefulness of our methods are: 1) the two studies were conducted in different time periods, and 2) the two studies had somewhat different focuses.

Following this comparison, we recommend that standardized, rapid bioassessment methods be developed for widespread use in Texas surveys. Previous attempts to develop simplified instream monitoring based on fish [50] and macroinvertebrates [113] should be considered in the development of methods for Texas. It may be possible to develop acceptable rapid bioassessment methods based on fish alone, thereby saving the extra expense and complexity of collection and identification of invertebrates. Simple and inexpensive surveys conducted at a greater number of sites may be more helpful than more complex surveys conducted at a smaller number of sites. However, more study will be required to develop the rapid bioassessment methods most appropriate for widespread use in Texas.

Impacts of Individual Hazardous Chemicals:

Very little information is available concerning how hazardous high body burdens of various individual toxic chemicals are to different species of fish and wildlife and the predator species consuming them. This makes it difficult to completely interpret residue data from studies such as this one. Therefore, we recommend more research be conducted to determine how hazardous body burdens of various individual contaminants are to fish and wildlife and to the predators consuming them.

Impacts of Mixtures of Toxic Chemicals:

Our findings indicate that fish and wildlife in polluted sections of the Trinity River typically carry complex mixtures of many different toxic chemicals. However, even less information is available on impacts of body burdens of mixtures of chemicals than on the impacts of individual chemicals. For example, there is a great need for more information on the effects of dietary exposure of predators to multiple PAHs [40] and to various combinations of organic and metallic contaminants.

Therefore, we recommend more research be conducted on the effects upon fish and wildlife and the predators consuming them of high body burdens of combinations of contaminants. These additional studies are necessary to more definitively assess the extent of synergistic, additive, or antagonistic effects.

Impacts Of Non-routine Contaminants:

Due to cost considerations and mode of action of some chemicals, we analyzed samples for only 67 contaminants. All were contaminants which we shall refer to as routine, since they are routinely available on standard laboratory scans commonly used by the Fish and Wildlife Service in fish residue studies. For purposes of this discussion, all other contaminants are defined as non-routine, although many of them may routinely occur in the Trinity River.

For perspective, there are over 62,000 chemicals cataloged by EPA in its Chemical Substances Inventory, many of which fish and wildlife might encounter in today's world.

We analyzed samples for routine contaminants known to accumulate in fish and wildlife tissues and for which reliable laboratory methods have been developed. Some routine contaminants tend to accumulate in only certain types of tissues, so we did not look for these contaminants in all tissues collected.

Some non-routine contaminants are metabolized quickly once inside fish and wildlife tissues. Other non-routine contaminants, including many of the recently developed pesticides, are acutely toxic and kill or injure fish and wildlife rather quickly but do not accumulate or persist in the tissues of the individuals affected. In order to more fully understand the overall impacts of toxic chemicals on Trinity River fish and wildlife, we recommend additional studies be conducted to identify the extent to which these non-routine chemicals occur in the river and its sediments, and the extent to which they are impacting fish and wildlife resources.

There are some non-routine contaminants whose chemical characteristics suggest possible accumulation in fish and wildlife but for which reliable laboratory methods for analysis in fish and wildlife tissues have not yet been developed. Analyses for some other chemicals (such as dioxins and dibenzofurans) would have been appropriate but was too costly given the budget of this study. Dioxins include some of the most toxic synthetic compounds known to man and have received widespread publicity due to controversies related to agent orange and the Times Beach Superfund site. Dibenzofurans are closely related to PCBs as well as dioxins and often occur as contaminants in PCB mixtures [114]. Some experts believe that many of the mammalian or human effects which have ascribed to PCBs may actually be caused by chlorinated dibenzofurans [114].

Nitrogen-containing aromatic compounds (NCACs) constitute another group of toxic chemicals which might be included in future monitoring of the Trinity. Many NCAC compounds are carcinogenic, mutagenic, or otherwise very hazardous [124,126]. For example, quinoline and some of its isomers are mutagenic and carcinogenic [126]. Carbazole and several related compounds and many isomers of benzacridine are also carcinogenic [126].

As better methods become available in our analytical laboratories, we recommend that additional studies be conducted to quantify the extent to which non-routine contaminants are accumulating in (or otherwise impacting) fish and wildlife.

Illegal Discharges Into Storm Drains:

Preliminary findings of surveys conducted by the Fort Worth Health Department, and related indications in our study, indicate there may be a significant impact upon Trinity River fish and wildlife from illegal discharges into Dallas/ Fort Worth area storm drains. These discharges include: 1) raw sewage (which involves some hospital sewage and indirect sources of industrial wastes), 2) used motor oil from individuals and automotive businesses, and 3) direct discharges of industrial waste. For example, one high rise office building in downtown Fort Worth was found to be discharging all of its raw sewage into storm drains leading directly to the Trinity River.

We believe the problem of illegal discharges into storm drains warrants serious consideration by municipalities and regulatory agencies. The innovative work of the Storm Drain Team of the Fort Worth Health Department provides a simple and economical example of a "first step" system for identifying and eliminating illegal discharges to storm drains. Other municipalities could use the Fort Worth program as a model for developing programs specific to their needs. There are some indications that the storm drain clean-up effort in Fort Worth may have already resulted in some relief to fish and wildlife in certain portions of the Trinity.

Another positive development related to storm drains is the fact that the Texas Water Commission, the North Central Council of Governments, and the Environmental Protection Agency are now looking closer at urban runoff problems. However, to insure the problems are adequately addressed, we recommend that intensive areawide studies of storm water discharges be conducted in Dallas/Fort Worth to more definitively quantify the relative impact of pollution from storm drains versus pollution from sewage plants and other sources.

Municipal Landfills:

We recommend that a study be undertaken to determine the extent of contamination of the Trinity River by toxic chemicals leaching, leaking, running off, or washing out of municipal landfills located in floodplains. Numerous recent reports have indicated that municipal landfills typically leak a wide variety of toxic and carcinogenic chemicals [46,80, 85]. This potential problem may be important in the Dallas/Fort Worth area due to the large number of municipal landfills which have been built in the floodplain of the Trinity and its tributaries.

Due to their chemical composition, many municipal landfill leachates are potentially as harmful as some of those from industrial landfills, yet the standards to insure toxic chemicals are not released into underlying waters are less stringent for municipal landfills [85]. A recent report by a researcher at Texas A.& M. documented that toxic and cancer causing chemicals were found in the leachate of all 58 landfills studied and that the leachate of some of the municipal landfills appeared to be as potent as the leachate from the highly-publicized Love Canal industrial landfill [85].

It is difficult to detect non-approved chemicals in the huge number of plastic bags which arrive at municipal landfills daily. In the compaction of the landfill, the plastic bags and many of the containers in them are crushed. In the underground environment of a municipal landfill, many plastics, pharmaceuticals, and paints degrade into chemicals which may be even more toxic than the products from which they originated [85].

Leachates of municipal landfills contain many solvents, trihalomethanes, and industrial chemicals which do not tend to accumulate in fish or wildlife tissues but which are directly toxic, carcinogenic, or mutagenic [85]. Municipal landfills have served as a repository for: 1) a great variety of hazardous chemicals used in households, 2) some hospital and infectious wastes, 3) a multitude of hazardous chemicals used by small businesses and legally disposed of under provisions for small-quantity (100 kg or about 25 gallons per month) generators [99], and 4) toxic chemicals or contaminated materials illegally dumped by large-quantity generators [85]. Under some circumstances, municipal landfills are permitted to accept old underground gasoline storage tanks and moderately contaminated soils dug up with them.

In addition to toxic chemicals, leachate from municipal landfill can have biological oxygen demand (BOD) concentrations higher than is typical for raw sewage or industrial waste [80]. Some municipal landfill leachates have been found to have BOD concentrations 85 times higher than concentrations typical of sewage sludge [80].

Some states have laws which prevent building municipal landfills in floodplains. One state, Michigan, has classified all municipal landfills as state-listed superfund sites.

A recent analysis by the North Central Texas Council of Governments revealed that current and past municipal landfills built in the floodplains of Dallas/Fort Worth were so numerous that one could generate a map of the Trinity and many of its tributaries by connecting dots representing landfill localities (Sam Brush, personal communication).

Prior to the mid 1970's municipal landfills in Texas were largely unregulated and permeable soils, the worst option from a leakage standpoint, were sometimes recommended rather than discouraged. Under current Texas law, large new landfills in floodplains are typically required to have some type of liner. Some are required to have slurry walls and dikes. On August 24, 1988, as this report was being finalized, the Environmental Protection Agency announced a proposal to more strictly regulate new municipal landfills.

However, these new requirements will not totally correct all the problems with currently operating landfills. Another problem is that the unregulated municipal landfills of the past have more potential for contamination of the environment than most currently operating landfills.

Sudden rainfall events may increase nonpoint source runoff from the numerous older landfills along the Trinity. Flood events also intensify the natural tendency of rivers to change course and cut into new banks. Thus the potential exists for episodic erosion of old landfills, in addition to the continuous potential for leaking, leaching, and runoff. The Trinity River floodplain contains many sandy areas which are very permeable and would speed the movement of leachates into underground flow of the river (which can resurface downstream) or into groundwater.

The potential for toxic chemical contamination of the Trinity River is also enhanced by the fact that the Dallas/Fort Worth area (unlike many other parts of the country) makes few options available to citizens to conveniently recycle or properly dispose of used motor oil, containers of pesticides, solvents, and other sources of hazardous chemicals. Some of these substances currently end up in municipal landfills [85]. A large number of the hazardous chemicals we found to be elevated in fish and wildlife in the Trinity River are typically found in leachates from municipal landfills [80,85].

The importance of municipal landfills as a potential source of hazardous chemicals is underscored by the fact that several municipal landfills have become federally-listed superfund sites. Most municipal landfills have some potential for eventually leaking contaminants into adjacent or underground waters [80]. Therefore, the use of floodplains for landfills, particularly the unregulated municipal landfills of the past, may be one source of toxic contaminants in the Trinity River.

The concentrations of toxic and oxygen-demanding contaminants entering the river from most municipal landfill sites may be low after being diluted by the river, especially during high flow conditions. However, the sheer number of these sites in the floodplains of Dallas/Fort Worth could result in a substantial cumulative impact on a small river like the Trinity, especially during the low flow conditions which typically precede a fish kill. Continuous (yet small) sources of contaminants can have long term impacts because of the persistent nature of chemicals like PCBs and mercury and their potential for causing repeated impacts as they move downstream. These are complicated issues, and we recommend that additional study be conducted to determine the extent of the potential impacts of municipal landfills on fish and wildlife resources in adjacent rivers.

We are concerned that most of the recently proposed landfills in the Dallas/Fort Worth area continue to be located in Trinity River floodplains. Of special concern is the long term necessity to inspect and maintain levees and liners of landfills in floodplains to assure permanent protection of water quality. Recent experiences at hazardous waste sites in the U.S. reveals that many of the single liners used at hazardous waste landfills have developed leaks [100]. It was this tendency to develop leaks that necessitated requiring double liners at some hazardous waste sites.

Municipal landfills are not monitored as closely for leakage as hazardous waste landfills. Changing this to provide long term monitoring of levees, liners, and leakage of municipal landfills, to the extent necessary to insure the landfills never leak, would add an increased and continuing financial burden on municipalities. The recently proposed 30 years of post-closure care for municipal landfills will not be sufficient in erosion-prone environments adjacent to rivers.

Therefore, to insure maximum long term protection of fish and wildlife resources downstream, we recommend that municipalities place municipal landfills in nonpermeable areas of upland sites rather than in floodplains.

Illegal Landfills and Dumps:

Illegal dumping of hazardous chemicals, illegal landfills, and unauthorized trash dumps are common occurrences in the floodplains of the upper Trinity River. Hazardous chemicals can leak into the Trinity River from these unlawful sources in much the same way they can leak from the non-regulated municipal landfills of the past.

One area where past and recent illegal dumping has been evident to us is the area in Dallas between Linfield street and South Loop 12, just upstream of our site number 9. This area is of interest because of the high contaminant levels we found just downstream. However, in the same general area or just upstream there are also 1) several floodplain-located municipal landfills which accepted industrial waste and were closed before municipal landfills were required to have liners [103], 2) urban runoff from much of Dallas/Fort Worth, and 3) the largest sewage plant in Dallas (Dallas Central Sewage Treatment Plant). With so many potential sources of pollution in the area and a substantial dilution factor in the river, it would be very difficult to design a study which would isolate and quantify only those contaminants entering the river from individual landfills. Nevertheless, we recommend that future surveys of toxic chemicals in the upper Trinity River attempt to provide as much insight as possible into the relative contributions of hazardous chemicals from illegal dumps. Upstream of Dallas/Fort Worth, where the river is less diluted by large volumes of treated sewage and where there are fewer pollution sources, it may be feasible to design and execute a study which would isolate the contaminant contributions of an illegal landfill or dump.

Aluminum:

We could find relatively little information on the potential impacts of excess aluminum on fish and wildlife. We recommend additional study be devoted to more definitively determine if elevated concentrations of aluminum are harmful to fish and wildlife. Most recent research on aluminum as a contaminant appears to be concentrating on issues related to acid rain and alzheimers disease. More work also needs to be done to more definitively determine if sewage treatment plants play a role in elevated aluminum concentrations in fish and wildlife downstream of Dallas.

Lake Livingston:

One of our findings is that several contaminants, including aluminum, beryllium, cadmium, chromium, iron, mercury, nickel, dieldrin, and *cis*-chlordane, showed a tendency to increase from upstream to downstream. Therefore, we recommend that a study be conducted to determine if the sediments and fish and wildlife of Lake Livingston are serving as the ultimate repositories for these contaminants.

Chlorine and Sewage Treatment Plants:

Since our 1985 collections, several of the large sewage plants in the Dallas/Fort Worth area have made improvements in the quality of the effluents they discharge into the Trinity River. This factor, in addition to favorable flow conditions in 1986-87, may have diluted or washed out contaminants and thereby benefited fish populations in the Trinity River.

However, the continuing discharge of large amounts of chlorine by the sewage plants remains a hazard to fish and wildlife in the upper Trinity River, especially during low flows (see detailed discussion in section on chlorine impacts).

Therefore, we recommend that municipalities, the Texas Water Commission, and the Environmental Protection agency continue their current efforts to identify alternative disinfection technologies for sewage treatment plant discharges. Since chlorination followed by de-chlorination is one of the technologies being considered, we recommended that strict effluent limitations be placed on chlorine discharges. For adequate protection of fish and wildlife resources, chlorine discharges should be limited to amounts which will not result in chronic instream levels of chlorine exceeding 2-11 ug/l [104,105].

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APPENDIX 1

Detailed Descriptions of Site Locations

Sampling Sites	River Mile	Location
1-MC	545.8	Mustang Creek M*100(W)
2-VSB	531.0	Clear Fork at Vickery Street Bridge M*76/(J)
3-OOD	529.2	Clear Fork near Purcy Street Drain M*62(Y)
4-RDB	522.5	West Fork at Riverside Drive M*77(D)
5-820B	513.6	West Fork at I-820 M*66(K)
6-WOVC	507.3	West Fork W. of Village Creek Confluence M*67(R)
7-GH	483.4	West Fork at Gifford Hill Bridge M*41B(P)
8-CCC	471.6	Trinity River at Cedar Crest Blvd. M*56(A)
9-12B	466.1	Trinity River at S. Loop 12 M*57(W)
10-5W	462.3	Trinity River 1 mile N. of I-20 M*68(B)
11-635B	460.3	Trinity River 1 mile S. of I-20 M*68(R)
12-MB	449.2	Trinity River at Malloy Bridge M*90(A)

*Page (with area location in parenthesis) number in Dallas or Fort Worth "MAPSCO" Map Books.

Detailed Descriptions of Site Locations

Sampling Sites	River Mile	Location
13-85B	408.5	Trinity River at FM85-Henderson County
14-TB	292.9	Trinity River 2 Miles South of Hwy. 79 Bridge
15-EAF	NA	East Fork at Malloy Road Bridge M*70A(V)
16-ELF	484	Elm Fork (California Crossing to Loop 12 M*22(X)
17-MTC	483.4	Mountain Creek at I-30 M*42(S)
18-IOD	NA	Purcy St. Storm Drain Canal, Ft. Worth M*62(Y)
19-SC	NA	Sycamore Creek at Confluence with West Fork, Ft. Worth M*77(D)
20-JC	NA	Johnson Creek at Waggoner Park, Grand Prairie M*41(P)
21-MP	NA	Pond between I-30 and Chippawa Drive, West Dallas M*42(S)
22-MS	NA	Slough Behind Metal Platers Area, Progressive Drive, West Dallas M*42(S)

*Page (with area location in parenthesis) number in Dallas or Fort Worth MAPSCO Map Books.

Detailed Descriptions of Site Locations

Sampling Sites	River Mile	Location
23-UD	NA	Port Worth Storm Drain 14, Just E. of Univ. Drive, South bank of river M*62(T)
24-287	353	Trinity River near Hwy. 287
25-RSB	NA	"Ranch Style Beans" Creek Just E. of Riverside Drive Bridge, M*77(C)
26-GC	541.8	Clear Fork Just Below Benbrook Dam M*87(V)
27-WF	533	West Fork E. of Ansley and Dennis Streets M*61(W)

*Page (with area in parenthesis) number in Dallas or Fort Worth "MAPSCO" Map Books.

APPENDIX 2

OBSERVATIONS ON TOXICITY IN THE TRINITY RIVER RESULTING FROM CHLORINATION OF SEWAGE TREATMENT PLANT EFFLUENT

Kirk E. Dean

ABSTRACT

In order to gauge toxicity resulting from the chlorination of sewage treatment plant effluent, we ran several in situ acute toxicity tests below the Village Creek Wastewater Treatment Plant in northeast Fort Worth. Single tests were also run near the Trinity River Central Plant in West Dallas and the Dallas Central Sewage Treatment Plant. Our tests followed the procedure described by Black and Burks (1986). Results of the tests show that the acute toxicity to fish may persist several miles below the plant's outfall to the river.

Due to much recent concern over the Trinity River fish kills, we planned to gauge the extent of toxicity resulting from the chlorination of sewage treatment plant effluent. We hoped to measure chlorine toxicity with the river in several flow stages, including a "rise" event resulting from a heavy rainfall. Most of the fish kills have been associated with these river rises.

Studies have shown that chlorine is acutely toxic to freshwater fish and invertebrates at concentrations from 710 ug/l down to 28 ug/l (U.S.E.P.A., 1986). The Environmental Protection Agency's freshwater acute toxicity criteria for chlorine is 19 ug/l; the chronic toxicity criteria is 11 ug/l. There are indications that the EPA will enforce some form of chlorine limitation in the future. In order to meet these criteria, sewage treatment plants will have to change their present disinfection methods, through dechlorination or alternative disinfection methods.

We used in situ acute toxicity bioassays with fish, along with total residual chlorine measurements, to determine toxicity. Though we believed that chlorine would be the primary toxicant in the river below the sewage treatment plants, the bioassays might show other toxic effects.

The toxicity tests were run in the Trinity River near Village Creek Wastewater Treatment Plant in northeast Fort Worth in the spring and summer of 1987. This plant treats about 100-125 million gallons of sewage per day, and its effluent often composes over half of the river's flow below its outfall, like many north Texas sewage treatment plants. We also ran several tests in the unchlorinated secondary effluent, to check for any inherent toxicity. Plant personnel assisted me by locating good test sites, obtaining some dissolved oxygen data, and supplying their daily records on effluent flow, total suspended solids (TSS), biochemical oxygen demand (BOD), ammonia, and residual chlorine. In addition, we did one toxicity test at the Trinity River Central Sewage Treatment Plant in West Dallas and one at the Dallas Central STP.

The toxicity tests followed as closely as possible the procedure described by Black and Burks in "Protocol for in situ Acute Toxicity Testing with Organisms." For these bioassays, mortality of test organisms is checked at one or more sites downstream from a suspected pollutant source, and compared with mortality at a control site upstream of the source. If there is high mortality downstream, compared to very little or no mortality upstream, then the toxicity of the influent has been demonstrated. This low budget bioassay can be used for pollutant-source screening, before or in conjunction with expensive chemical analyses. No technical expertise is required. Floating plastic "trolling" minnow buckets are used as containers when the test organisms are fish.

We chose Golden Shiners, *Notemigonus crysoleucas*, as the test organism. They are indigenous to the Trinity River, and we believed they would be sensitive to pollution since they are present in the river's tributaries west of downtown Fort Worth, but absent in the more polluted sections east of downtown (Roy Irwin, personal communication). They are also easily obtainable from a bait shop. Two different bait shops supplied the minnows, but no difference in mortality rates was obvious. The minnows were transported to the test sites in a large ice chest full of river water, with aeration. They were handled carefully, but appeared to be very hardy. The minnows for the control site were placed last, so they had to endure the most transport stress. The test results were discarded as inconclusive if greater than 10% of control organisms died during the test period.

Two minnow buckets containing ten fish each were placed at the control site and four other sites downstream from the STP outfall. The control site was about 300 feet upstream from the outfall. Site No. 1 was about 100 feet below the outfall; sites 2,3 and 4 were 0.8, 1.7, and 5.1 miles downstream of the outfall. Dissolved oxygen was measured inside the minnow buckets and outside in the river to ensure that this was not a factor contributing to mortality. Water temperature was also measured. Total chlorine was measured with a Hach kit. The tests lasted for the full 96 hour acute toxicity period or until 100% of the downstream organisms died.

The Fathead Minnow, *Pimephales promelas*, is the fish used for most laboratory toxicity bioassays. In order to compare the Golden Shiners to Fathead Minnows as test organisms, eight week old Fathead Minnows were obtained from Scott Dyer and Ken Dickson at North Texas State University, and a test was run in August with each species in containers side-by-side at several sites. The containers had to be modified so that the tiny Fathead Minnows could not escape through the vent holes. Fiberglass window screen and non-toxic adhesive were used for this purpose.

Test results were analyzed by statistical techniques described by Black and Burks (1986) in their protocol. Cumulative percent mortality is plotted versus time on logarithmic probability paper. After drawing a straight line through the points, and testing its goodness of fit with a chi-square test, the median lethal time (LT_{50}) can be read from the graph, and 95% confidence intervals can be calculated.

<u>Test Results</u>

Three toxicity tests were run in wastewater that had been treated physically in primary treatment, and biologically in secondary treatment, but had yet to be filtered and chlorinated before discharge. From 7-11 April, no mortality was recorded (n=60). From 21-15 May, 10% mortality of test organisms occurred over 96 hours (n=20). From 27 June - 1 July, 20% mortality was recorded (n=20). The mortality that occurred could have been due to the lack of shade or the extremely turbulent conditions in the canal. However, based on our results, we cannot rule out at least occasional toxicity of STP effluent before chlorination.

Table #1
GOLDEN SHINER MORTALITY IN UNCHLORINATED SECONDARY EFFLUENT
VILLAGE CREEK WASTEWATER TREATMENT PLANT

	#Fish	96-Hour Percent Mortality	Temp. Celcius	Dissolved Oxygen ppm	Mean Effluent Flow mgd	Mean Effluent Ammonia mg/l	Mean BOD lbs/day	Mean TSS lbs/day
7-11 April	60	0%	20	6.1	108.6	0.46	1086	1812
21-25 May	20	10%		6.7	121.1	0.14	1011	1528
27 June-1 July	20	20%			120.4	0.31	1138	2224

Investigator concerns over poor water circulation and reduced dissolved oxygen in the test containers were unfounded. Dissolved oxygen levels inside and outside the minnow buckets did not appear to be different. The very high survival rate of the test organisms at the control site, at least until mid-summer, indicated that the containers were adequate and non-toxic.

Table #2
DISSOLVED OXYGEN CONCENTRATIONS WITHIN AND
OUTSIDE THE MINNOW BUCKETS

	D.O. River ppm	D.O. Bucket ppm		D.O. River ppm	D.O. Bucket ppm
21 APRIL			25 April		
CONTROL	8.5	8.5	CONTROL	11.2	10.9
#1	7.6	7.6	#1	8.6	8.5
#2	7.2	7.3	#2	8.6	8.6
#3	7.1	7.1	#3	9.2	8.9
23 APRIL			11 May		
CONTROL	10.5	10.3	CONTROL	7.9	7.9
#1	8.2	8.1	#1	7.4	7.5
#2	8.6	8.6	#2	7.4	7.3
#3	9.2	8.3	#3	7.2	6.9
MEAN	8.35	8.27			

In the acute toxicity tests in the river below the Village Creek plant outfall, the highest measured toxicity occurred on April 21, when total residual chlorine levels of 0.6 mg/l were recorded at site #4, 5.1 miles downstream of the outfall. This was probably due to low flow on the river (23.0 mgd at Nutt Dam), high chlorine concentration of STP effluent (2.0 mg/l) and a large amount of ammonia in the effluent (1.3 mg/l). Ammonia can help keep chlorine from leaving the water as a gas (Manahan, 1984). Toxicity from this pulse of chlorine might affect the fish and invertebrates far downstream. (See Table #3)

Successful tests were also run on 23 April, 25 April, 11-14 May, and 9-13 August. Significant control mortality occurred in the latter test before the 96 hour period was over, but after all test organisms at sites 1, 2 and 3 had died. The mortality of the control organisms may have been due to handling stress, stress at the bait shops where they were

Table #3

Golden Shiner Mortality Below Village Creek Wastewater Treatment Plant Outfall - Successful Tests

	LT50 Hours w/95% confidence level	Mean Total Chlorine mg/l	Mean Temp.	D.O. River ppm	Sewage Treatment Plant Data: Mean Effluent Total Chlorine mg/l	Mean Effluent Ammonia mg/l	Mean BOD lbs/day	Mean TSS lbs/day	Mean Daily Effluent Flow mgd	Mean Trinity River Flow at Nutt Dam mgd
'21 April					2	1.3	5194	1731	103.8	23
Control	*			8.5						
#1	<1.0	0.9	21.6	7.6						
#2	<1.0	1.3	22.3	7.2						
#3	<1.0	1.2	21.2	7.1						
#4		0.6								
'23 April					1.1	0.1	1767	883	105.9	23
Control	*	<0.1	22.8	10.5						
#1	<1.0	0.9	22.8	8.2						
#2	<1.0	0.8	22.9	8.6						
#3	1.67 ± 0.16	0.8	22.8	8.4						
'25 April					1.3	0.3	879	879	105.4	27
Control	*	<0.1	23.5	11.2						
#1	1.50 ± 0.15	0.6	23.6	8.6						
#2	2.17 ± 0.22	0.6	23.5	8.6						
#3	7.92 ± 0.82	0.4	24.3	9.2						
'11-15 May					1.4	0.2	1380	2068	110.3	79.2
Control	*	<0.1	25.7	7.9						
#1	7.70 ± 0.91	0.5	24.8	7.4						
#2	17.0 ± 0.41	0.5	24.9	7.4						
#3	38 ± 3.62	0.3	24.9	7.2						
'9-13 August					1.4	0.36	899	899	108	16
Control	26 ± 4.9	<0.1	31.3	7.2						
#1	<1.0	0.4	29.7	7.3						
#2	<1.0	0.4								
#3	1.75 ± 0.26	0.3	29.6	7.4						
#4	49 ±13.3	0.1	29.1	7.4						

*No significant mortality -<=10% over 96-hour Acute Toxicity Period

Control - 300 Ft. upstream of outfall

#1 - 100 Ft. downstream of outfall

#2 - 0.8 miles downstream of outfall

#3 - 1.7 miles downstream of outfall

#4 - 5.1 miles downstream of outfall

raised, or high water temperature (~30°C). Dissolved oxygen in the river was still fairly high (~ 7.3 ppm). It is possible that, due to low flow on the river, chlorinated STP effluent flowed upstream and contaminated the control site. No increase in chlorine concentration was measured, however.

Toxicity tests on 21-25 May and 27-1 July were unsuccessful. These tests were to concentrate on the toxicity farther downstream, at sites 3 and 4 only. The latter test was ruined when one pair of minnow buckets were stolen, and another pair was used by someone for target practice. A rapid rise in river level on May 24-25 made two pair of containers unaccessible, then left one pair high and dry as it receded. Another pair was stolen. For the one pair of containers that survived the river rise, no excessive mortality was noticed. The unsuccessful tests occurred when there was relatively low chlorine toxicity in the river.

Overall, the mortality rate of the test organisms appeared to be closely related to the chlorine concentrations measured in the river during the tests. We observed rapid mortality of test organisms at chlorine concentrations greater than 0.3 mg/l; this high toxicity decreased to almost no acute toxicity below 0.1 mg/l. We could not find any strong correlation between toxicity and river flow or the sewage tratement plant's daily average effluent parameters such as BOD, total suspended solids, ammonia, or chlorine.

The comparison between Golden Shiners and Fathead Minnows as test organisms yielded interesting results. Though conclusions cannot be made from one test, it appeared that the Fathead Minnows died very quickly (LT_{50} < 1 hr.) at chlorine concentrations of 0.3-0.4 mg/l, but survived well in ambient river conditions at the control site and at site #4, where Golden Shiners were dying. This effect may be due to our use of juvenile Fathead Minnows vs. adult Golden Shiners. The Fathead Minnows were put through more transport stress in their trip from Denton, but they did not seem to be adversely affected by it.

Table #4

COMPARISON BETWEEN GOLDEN SHINERS, *Notemigonus crysoleucas*, AND FATHEAD MINNOWS, *Pimephales promelas*, AS TEST ORGANISMS

MORTALITY BELOW VILLAGE CREEK STP OUTFALL

9-13 August	Control		#1		#2		#3		#4	
Species	N.c.	P.p.	N.c.	P.p.	N.c.	P.p.	N.c.	P.p.	N.c.	P.p.
Time(hours)										
1	0	0	90%	100%	80%	100%	40%	100%	0	0
2	0	0	100%		100%		40%		0	0
4	10%	10%					90%		0	0
8	10%	10%					100%		0	0
24	40%	10%							30%	10%
48	80%	10%							50%	10%
72	100%	gone							60%	10%
96									70%	10%
LT_{50}(Hours)	25±6.4	*	<1.0	<1.0	<1.0	<1.0	1.8±0.23	<1.0	48±14.6	*
D.O.(ppm)	7.2		7.3				7.4		7.4	
Temp(celcius)	31.3		29.7				29.6		29.1	
Total Chlorine (mg/l)	<0.1		0.4		0.4		0.3		0.1	

* - No significant mortality - < 10% over 96-hour acute toxicity period

The toxicity tests at Trinity River Central and Dallas Central sewage treatment plants were limited by poor accessibility to the river and by the travel time required to drive to Dallas. At Trinity River Central, an LT_{50} of 6.5 hours was measured for Golden Shiners about 100 feet below the outfall and 19 hours at 0.7 mile downstream. Black bullhead and Carp in stress were observed at the outfall to the river. At Dallas Central, access to the river below the outfall was practical only just below the outfall, and 2.5 miles downstream, at Loop 12. The only bioassay indicated substantial toxicity just below the outfall. Chlorine concentrations of 0.4 and 0.2 mg/1 were measured at Loop 12 on 10 and 26 July.

Table #5
GOLDEN SHINER MORTALITY IN THE TRINITY RIVER NEAR TRINITY RIVER CENTRAL STP AND DALLAS CENTRAL STP

Dallas Central STP	26 July		10 July
	LT_{50} hours	Total Chlorine mg/l	Total Chlorine mg/l
CONTROL-0.5 Miles upstream of outfall	*	<0.1	<0.1
100 ft. downstream of outfall	1 ± 0.1	1.0	1.7
2.5 miles downstream of outfall		0.2	0.4

Trinity River Central STP	25-27 July	
	LT_{50} hours	Total Chlorine mg/l
CONTROL-0.4 Miles upstream of outfall	*	<0.1
100 ft. downstream of outfall	6.5 ± 0.3	0.4
0.7 mile downstream of outfall	19.0 ± 2.4	0.3

* No significant mortality - <=10% over 96-Hour Acute Toxicity Period

Texas state water quality standards stipulate that there must be a zone of passage for aquatic organisms along the stream bank opposite the point where pollutants are discharged. In this zone there should be no toxicity. This allows free movement of fish up and downstream past the discharge. Sewage treatment plants on the Trinity River have a difficult time meeting this requirement, as the river flow is often very low compared to their effluent discharge. Based on the movement of foam from the outfalls, it appeared that there was no zone of passage at any of three plants visited. One measurement of chlorine concentrations across from the Village Creek STP outfall (0.4 mg/1), along with the mortality of test minnows supported the opinion that there was no zone of passage.

Conclusion

We have presented evidence that the chlorination of sewage treatment plant effluent causes significant toxicity to sensitive fish species in the Trinity River, sometimes five miles or more downstream. There exists the possibility that chlorine could play a role in causing the fish kills. During heavy rainfall when water inundates the treatment process, sewage treatment plants sometimes release incompletely treated or untreated sewage to the river, which may be heavily chlorinated as a safeguard.

We did not find very significant acute toxicity when river chlorine concentrations were below 0.1 - 0.2 mg/l.

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