CONTAMINANTS IN SELECTED OKLAHOMA WATERS: CORRELATION OF AROMATIC HYDROCARBONS WITH FERAL FISH HEALTH

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STATEMENT OF THE PROBLEM

Urbanization and industrial development along the world's coastlines has proceeded essentially unimpeded for hundreds of years. Commensurate with this, the chemical quality of adjoining waters and benthic sediments has been modified by continual discharges of hazardous solid and liquid wastes (3). As a result, significant incidences of fish die-offs, injuries, and diseases have been documented both world-wide and in the state of Oklahoma (1). Perhaps the most insidious of all potential diseases are cancers that frequently occur years after the initial carcinogen exposure (Reviewed in 2). Cancers in feral fishes have been identified in a variety of North American locations, all impacted by man, including but not limited to The Puget Sound, The Great Lakes, Boston Harbor, Hudson Bay, and near Los Angeles (2). Surprisingly, little attention has been directed toward fishes in the inland waters of the mid- and southwest United States, including Oklahoma, though much is known of the water and sediment chemistry. Oklahoma waters receive discharges from oil refineries, farmlands, and municipal wastes. Consequently, these waters have been documented to contain many mutagenic, teratogenic, and carcinogenic compounds (e.g., 1). Based on the weight of these and previous studies, it was both appropriate and necessary to examine closely resident feral fish populations, residing in polluted Oklahoma waters.

The mere identification of cancer, changes in population structure, etc. in fish(es) will be an important first step in assessing the overall quality of the ecosystem. However, based on results of similar studies in other waterways in North America, we can expect to find a myriad of potential mutagenic and

carcinogenic compounds. For example, in Puget Sound over 2,000 man-made pollutants have been identified (3). Consequently, it will also be worthwhile to conduct a preliminary assessment aimed at determining which class(es) of compounds were present that might be capable of impacting resident fish populations.

OBJECTIVES OF THE PROJECT

The long history of pollution in Oklahoma waters, coupled with recent studies of water and sediment chemistry, suggests a strong likelihood of fish populations being impacted (1). Consequently, fishes from a number of potential hazardous sites were collected and subjected to complete histological/microscopic examination. Concurrently, we conducted a complete chemical analysis of the water and sediments these animals resided in for possible contaminants.

METHODOLOGY

The following sites were identified as potential sites for impacted fish populations. It was not our intent to survey all Oklahoma waterways. Rather, we hoped to identify initially a single impacted site and an affected species. Subsequently, we will explore the causative aspects of impaction in greater detail through additional laboratory and field studies.

Sampling Sites

1. *Ardmore, Oklahoma*

Ardmore is approximately 180 miles south of Oklahoma City in the south central part of the state in Love county. Total Oil Refinery is located here with effluent drainage flowing into Sand Creek and eventually reaching the Washita River. The most recent reports show that the refinery is capable of processing

about 62,000 barrels of crude oil per calendar day (4). Sampling was conducted downstream from the refinery discharge (Station 1), upstream from the refinery discharge (Station 2), and at the point of discharge (Station 3). Station 1 was located in SE1/4 SW1/4 NE1/4 S16 T4S R2E. This was approximately 0.5 miles north of the southern boundary of the refinery. Station 2 was in SW1/4 SW1/4 S21 T4S R2E. This was approximately 0.5 miles south of the southern boundary of the refinery. Station 3 was approximately 0.5 miles north of station 2 in NE1/4 NW1/4 S21 T4S R2E.

2. *Cyril, Oklahoma*

Cyril is located in Caddo county in the southwestern part of the state approximately 90 miles southwest of Oklahoma City. This was the location of the Oklahoma Refining Corporation oil refinery until its close in the mid 1980's, at which time it was capable of processing about 15,000 barrels of crude oil per calendar day (5). Our focus at this site was Gladys Creek. It flows from the north, along the eastern boundary of the oil refinery and continues on to the south where it eventually reaches the Little Washita River. [The sampling stations are shown on the map as: Station 1, Station 2, Station 3, and Station 4.] Station 1 is named Brown Pond and is formed by the damming of Gladys Creek; it was located at NW1/4 NE1/4 S19 T5N R9W. Station 2 was 200 m below Brown Pond and had visible contaminated water leaching into the creek from the western banks. Station 3 was 800 m above Brown Pond at SW1/4 SW1/4 S18 T5N R9W and served as the reference station. Station 4 was 850 m below the southern oil refinery boundary at NE1/4 NW1/4 S30 T5N R9W and served as a downstream reference station.

3. *Okmulgee, Oklahoma*

Okmulgee is located in the east central part of the state in Okmulgee county. The Oklahoma Refining Company was operating an oil refinery here through the early 1980's. The refinery was capable of processing 24,000 barrels of crude oil per calendar day (5). The creek of interest, Okmulgee Creek, flows from the north, along the eastern refinery boundary, through Okmulgee and on south to converge eventually with the Deep Fork of the Canadian River. Okmulgee Creek is known as "Tar Creek" by the people of Okmulgee because of the presence of oil in the creek apparently from spills. Sampling was performed at three stations: 1) a reference station above the refinery site (Station 1) approximately one mile, NE1/4 NE1/4 S32 T14N R13E, at the state highway 52 bridge; 2) on the refinery site at the point of discharge (Station 2) beneath the bridge crossing from the processing area to the tank farm, SE1/4 SE1/4 S31 T14N R13E; and 3) below the site approximately one mile (Station 3), S1/2 SE1/4 S7 T13N R13E, below the 12th street bridge.

4. *Arkansas River, Tulsa, Oklahoma*

Tulsa is a large city in the northeastern corner of Oklahoma. The Arkansas River flows east through Tulsa from the west. There are two operating oil refineries located on the Arkansas River and a nonoperational third that has been declared an Environmental Protection Agency Superfund Site. The Sinclair Oil Company and the Sun Oil Company have crude oil processing capacities of 50,000 and 85,000 barrels per calendar day, respectively. As is typical with this type of industry, effluents are discharged, after inplant treatment, into a nearby waterway. Another major source of contaminants is the city's stormwater

drains. There are several large drains entering the river from the downtown area. Historically, stormwater runoff from large metropolitan areas has been shown to contain many contaminants such as those released by automobiles from the incomplete combustion of fossil fuel. The river averages around 0.5 kilometers in width and 2 to 3 meters in depth much of the year.

Sampling was conducted at four stations along the river. Stations 1 and 2 were at the discharge sites of the Sinclair refinery, S1/2 SW1/4 S13 T19N R12E, one on each bank of the river. Station 3 was between the Sinclair and Sun Oil refineries beneath the 12th street bridge, N1/2 NW1/4 S13 T19N R12E, and Station 4, which was the reference station, was several miles upstream, N1/2 SW1/4 S9 T20N R9E, in Keystone Lake.

5. *Arbuckle Lake*

Arbuckle Lake is located in the Arbuckle Mountains in south central Oklahoma in Murray county. This lake is located in an area where natural sulfur springs and asphalt formations occur in the typical hydrogeology. A preliminary survey (Jimmie Pigg, personal communication) revealed possible malignant neoplasms occurring in the feral gizzard shad population in this lake. As this was an unusual occurrence, it merited further study. The two sampling stations were at the Guy Sandy boat access on the northwestern side of the lake in N1/2 SW1/4 S7 T1S R3E. Station 1 was along the western shore of a northern arm named the Guy Sandy Arm because the inflow is from Guy Sandy Creek. Station 2 was in a small cove immediately to the west of the boat ramp. Alternate sampling stations were surveyed but produced almost no shad per net night; therefore, these stations were abandoned for the remainder of the study.

Sampling of Fishes

Sampling of fishes was restricted to abundant benthic and benthic-associated species including members of the catfish (i.e., channel catfish and bullhead), carp (carp and river carpsucker), and catostomid families. These species were chosen because most potential carcinogens are water insoluble (or slightly soluble) and tend to accumulate in the sediments. Benthic species spend their entire lives in close proximity to these sediments. Pelagic species were omitted from these studies as these species are not as likely to express cancer owing to their low exposure rate (relative to benthic species) to the water insoluble compounds.

Sampling of fish populations primarily involved electroshocking, gill netting, and dip netting. Individual fish were necropsied and, following gross visual examination of all major tissues, samples of liver, kidney, stomach, muscle, and spleen were collected for routine histological processing. H & E sections of tissues were subjected to microscopic examination to determine the nature and/or extent of malignancy.

Diversity indices have been widely used to express differences in two communities of organisms. Two measures of species diversity have come to be widely used by ecologists today. The Simpson's Diversity Index (6) was one of the earliest indices that included both the total number of species present and the relative abundance of each species. The equation of:

Equation 1:

$$D = \frac{\sum_{i=1}^{s} n_i(n_i - 1)}{n(n-1)}$$

where **D** is the Diversity, **s** is the total number of species, $\mathbf{n_i}$ is the number of individuals of the i^{th} species, and **n** is the total number of individuals, defines Simpson's Diversity Index. This was described by Krebs (8) as being the probability of randomly picking two organisms of different species.

Shannon's Diversity Index is another commonly used index. It is based on information theory and is centered on the concept of uncertainty. If there are very few species present, we can be fairly sure of which species a randomly sampled individual will be. The equation is:

Equation 2:
$$H' = \frac{(N \log N - \Sigma n_i \log n_i)}{N}$$

where **H'** is the diversity, **N** is the total number of individuals, and $\mathbf{n_i}$ is the number of individuals of the i^{th} species. The Shannon Diversity Index is most appropriately used where one is acquiring random samples from a larger community (8). Since the random sample probably does not contain representatives from all of the species present, the index is somewhat biased but not so much as to affect the diversity index.

Community similarity indices are used to quantify how two separate communities relate to each other. Two indices are the Percent Similarity and Morisita's Index (9). The Percent Similarity is a sum of the lowest percentages of the total number of individuals that a species represents. For example a species

that comprises 50% of one community and 22% of the other community would account for 22% of the total 100% similarity possible for two communities. This is based on total numbers of individuals and total numbers of species present. Morisita's Index is based on Simpson's Diversity Index. It is the probability that two individuals drawn from two communities will be from the same species. The formula is:

Equation 3:
$$I_M = \frac{2\Sigma x_i y_i}{(\tau_1 + \tau_2) N_1 N_2}$$

where x_i is the abundance of the i^{th} species in community one and y_i is the abundance of the i^{th} species in community two, τ_1 is the Simpson Diversity of community one and τ_2 is the Simpson Diversity of community two, and the N's are the total number of individuals from the respective communities. The value of the index ranges from 0 to around 1, 1 being the most similar.

When appropriate, the fish communities were compared using the Shannon Diversity Index and the Percent Similarity and Morisita's indices.

Sampling of Water

Three samples of four liters of water were taken at each station. Water was collected in pre-cleaned, four-liter amber bottles. Samples were stored in the dark at 4°C until the time of extraction. Four liters of water were extracted on a Carbon-18 (C18) bonded solid phase extraction column (Bond Elut, Analytichem International, Product #607306). The columns were conditioned by passing two column volumes (12 ml) of methylene chloride through under a slight positive pressure, followed by two column volumes of reagent-grade water. The columns

were not allowed to dry from this point on. The columns were then connected to a two-liter separatory funnel, and four liters of water were passed through under a slight vacuum. The columns were air-dried under vacuum for 15 minutes to remove residual water prior to elution. Compounds were eluted with 40 ml of methylene chloride. This eluate was then passed through a 5 g column of sodium sulfate to remove excess water and rinsed twice with 2 ml of methylene chloride. The eluate was then concentrated by rotary evaporation in a 60°C water bath to 15 ml, transferred to a 15 ml concentrator tube, and finally concentrated to 1 ml in a 60°C water bath with nitrogen purge. The sample was then analyzed by gas chromatography/mass spectrometry. A VG Analytical TS-250 mass spectrometer connected to a Hewlett-Packard 5890A gas chromatograph was used for the analysis. A 30-meter, 0.32-mm inner diameter capillary column with an SE-54 bonded phase was used in the chromatograph. One to three microliters, depending on concentration, was injected and the sample was subjected to a programmed temperature gradient that raised the temperature 10°/minute from 50° to 280° C. The spectra of each peak were compared to reference spectra contained in the NIST on-line library (10).

Sampling of Sediments

Sediment samples were collected at the locations described above. Three replicate samples of one liter each were taken in pre-cleaned amber bottles with teflon lid liners (Scientific Specialties Service, B71132). The samples were stored at -40°C until extraction. In pilot studies, two separate methods of sediment extraction were employed. One was used by Malins et al. in studying Puget Sound sediments (11) and the other by Fabacher et al. in studying Black River sediments

(12). These methods were compared for their ability to resolve compounds and the better of the two, that which provided the best separation of compounds observed in our studies (12), was used with minor modifications as described below.

The sediments were thawed and air-dried under a hood for 24 hours prior to extraction. A 100 g aliquot of sediment was then powdered in a blender (Hamilton Beach, model #585-3). The sediment was extracted two times with 100 ml of benzene:methanol (60:40) and two times with 100 ml of methylene chloride. At each step, the slurry was shaken for two hours at 400 rpm on an orbital shaker. The sediment was allowed to settle out for 20 minutes, and the solvent was then decanted off the top into a 500 ml solvent rinsed bottle. The extracts were concentrated with a rotary evaporator in a 60°C water bath to about 10 ml. This solution was transferred to a 15 ml concentrator tube with two, two-ml rinses of methylene chloride. Finally, each sample was concentrated at 60°C under a continuous nitrogen stream to one ml. The sample was then considered ready for fractionation on an alumina column.

An 11 x 250 mm glass chromatography column with a 200 ml solvent reservoir was fitted with a glass wool plug and filled with 9 g of neutral alumina activated at 200°C for 12 hours. Enough N-hexane was added to cover the alumina, and the solution was shaken to remove any air bubbles. A small layer of sand was placed on top of the alumina to prevent disturbance when the sample or solvent was added. The sample was applied and eluted with 400 ml of N-hexane. This first fraction contained mostly aliphatic hydrocarbons. The column was then eluted with 1000 ml of benzene. This fraction contained mostly aromatic hydrocarbons. Finally, the column was eluted with 1550 ml of chloroform. This

final fraction contained mostly the nitrogen containing aromatic hydrocarbons. All three fractions were reduced by rotary evaporation to about 15 ml, and resultant solutions were concentrated on a 60°C water bath under a nitrogen stream to one ml. The samples were then analyzed by gas chromatography/mass spectrometry. A VG Analytical TS-250 mass spectrometer was used, with the same parameters as described earlier for the water analysis. One to three microliters, depending on concentration, was injected and the sample was subjected to a programmed temperature gradient (10°/minute from 50° to 280° C). The spectra of each peak were compared to reference spectra contained in the NIST on-line library for compound identification (10).

FINDINGS

Results of Water and Sediment Sampling

1. Ardmore, Oklahoma

A total of 13 compounds were identified in the water and sediment samples (Table 1). The Station 1 water sample had one compound: benzyl butly phthalate. The Station 1 sediment sample had seven compounds. Decamethylcyclo-pentasiloxane is suspected to be an artifact arising from silicone stopcock grease used on the glassware. The Station 2 water sample was free of compounds. The sediment sample contained three compounds: 1,3-dimethylbenzene, 2,3,4-trimethyl-1,4-pentadiene, and 2,3,3-1,4-pentadiene.

The Station 3 water sample contained three compounds: benzyl butyl phthalate, 2,1,1-(1,1-dimethylethyl)propanoic acid, and 2,2-dimethyl-1,2-diphenylethanone. The sediment sample contained four compounds: 1,3-dimethylbenzene, (1-methylethyl)benzene, 2,3,4-trimethyl-1,4-pentadiene, and

decamethylpentasiloxane. The decamethylcyclopentasiloxane is suspected to be an artifact from the silicone stopcock grease used on the glassware.

2. *Cyril, Oklahoma*

Water and sediment samples contained 24 compounds (Table 2). Water from Station 1 revealed no compounds. Station 1 sediment contained five compounds: 5,6,7,7a-tetrahydro-4,7,7a-trimethyl-(S)-2(4H)-benzofurenone, 1,3-dimethylbenzene, 4-hydroxybenzenesulfonic acid, methylbenzene, and ethylbenzene.

TABLE 1. Ardmore Water (W) and Sediment (S) Analysis

Compound	Station 1(D)	2(U)	3(I)
Benzyl butyl phthalate	W		W
2,6,10,14-Tetramethylhexadecane	S		
1,3-Dimethylbenzene	S	S	S
Propylbenzene	S		
(1-Methylethyl) benzene	S		S
1-Ethyl-3-Methylbenzene	S		
1,4-Diethylbenzene	S		
2-Ethyl-1, 4-dimethylbenzene	S		
2,3,4-Trimethyl-1, 4-pentadiene		S	S
2,3,3-Trimethyl-1, 4-pentadiene		S	
2-1,1-(1,1-Dimethylethyl) propanoic acid			W
2,2-Dimethyl-1, 2-Diphenylethanone			W
decamethylpentasiloxane			S

(D = downstream, U = upstream, I = impacted)

Station 2 water contained six compounds as was expected due to visible contamination leaching into the water from the creek banks. Station 2 sediment contained 12 compounds. Water from Station 3 contained no compounds above detection limits. Sediment contained two compounds: ethylbenzene and 2,6,10-trimethyldodecane.

Water from Station 4 contained no compounds above detection limits. Station 4 sediment contained one compound: α, ß-dimethylbenzeneethanol.

Water samples taken from all stations for BTEX analysis revealed that none of these compounds was present above detection limits (<0.005μg/l) at any station.

3. *Okmulgee, Oklahoma*

A total of 17 compounds were positively identified in the water and sediment samples (Table 3). Water analysis at Station 1 revealed no compounds above detection limits. Sediment samples from Station 1 contained 12 compounds.

No compounds were seen above detection limits in the water at Station 2. Station 2 sediment contained two compounds: 2,3,5-trimethylphenanthrene, and 1,1-dichloro-2,2-difluoroethane.

Station 3 water contained one compound: 3,5-dimethylcyclohexanol. Station 3 sediment contained three compounds: diethyl phthalate, phehanthrene, and 2,3,4-trimethyl-1,4-pentadiene.

TABLE 2. Cyril Water (W)and Sediment (S) Analysis

Compound	Station 1(P)	2(I)	3(U)	4(D)
5,6,7,7a-Tetrahydro-4,7,7a-trimethyl-(S)-2(4H)-benzofuranone	S			
1,3-Dimethylbenzene	S			
4-Hydroxybenzenesulfonic acid	S			
Methylbenzene	S			
Ethylbenzene	S		S	
2,4-Dimethyl-2,3-heptadien-5-yne		W		
1-Methyl-2-(1-methylethyl)benzene		W		
1,2,3,4-Tetramethylbenzene		W,S		
1,2,3,5-Tetramethylbenzene		W		
1-Methylnaphthalene		W		
1,5-Dimethylnaphthalene		W,S		
2-Ethyl-1,4-dimethylbenzene		S		
1-Ethyl-3,5-dimethylbenzene		S		
Diethylmethylbenzene		S		
Ethyl-1,2,4-trimethylbenzene		S		
2,4-Dimethyl-1-(1-methylpropyl)-benzene		S		
Pentamethylbenzene		S		
1,2,3,4-Tetrahydro-1,5,8-trimethylnaphthalene		S		
1-Ethylnaphthalene		S		
1,6-Dimethylnaphthalene		S		
1-Bromo-4-(2-phenylethyl)benzene		S		
2,6,10-Trimethyldodecane			S	
α,ß-Dimethylbenzeneethanol				S

(P = pond, I = impacted, U = upstream, D = downstream)

TABLE 3. Okmulgee Water (W) and Sediment (S) Analysis

Compound	Station 1(U)	2(I)	3(D)
1,2,3,4-Tetramethylbenzene	S		
Naphthalene	S		
1-Ethylidene-1H-indene	S		
Diethyl phthalate	S		
Benzyl butyl phthalate	S		
1,3-Dimethylbenzene	S		
Propylbenzene	S		
(1-Methylethyl)benzene	S		
1-Ethenyl-2-methylbenzene	S		
1,2-Diethylbenzene	S		
1-Ethyl-2,3-dimethylbenzene	S		
(1-Methylpropyl)benzene	S		
2,3,5-Trimethylphenanthrene		S	
1,1-Dichloro-2,2-difluoroethane		S	
3,5-Dimethylcyclohexanol			W
Phenanthrene			S
2,3,4-Trimethyl-1,4-pentadiene			S

(U = upstream, I = impacted, D = downstream)

4. *Arkansas River, Tulsa, Oklahoma*

A total of 17 compounds were identified in the water and sediment samples (Table 4). Water from Station 1 contained three compounds: 4,5-dimethylnonane, 2-methyl-1-(1,1-dimethylethyl)propanoic acid, and diethyl phthalate. Sediment contained one compound: α,ß-dimethylbenzeneethanol.

Water from Station 2 contained two compounds: 2-methyl-1-(1,1-dimethylethyl)-propanoic acid and diethyl phthalate. Sediment contained 7

compounds: 1-methylnaphthalene, 1,5-dimethyl-naphthalene, 2-methylnaphthalene, anthracene, 9-octadecen-1-ol, pyrene, and 2,6,10-trimethyldodecane.

Water from Station 3 contained two compounds: 2-methyl-1-(1,1-dimethylethyl)propanoic acid and decamethylpentasiloxane, which is suspected to be an artifact from the silicone stopcock grease used on the glassware. Sediment contained 6 compounds: 1-methylnaphthalene, pyrene, 2,6,10-trimethyl-dodecane, phenanthrene, benzo[a]pyrene, and methylbenzene.

Water from Station 4 contained no compounds above detection limits. Sediment contained two compounds: 12-(acetyloxy)-methyl ester 9-octadecenoate and (1-methylethyl)-benzene.

5. *Arbuckle Lake, Murray County, Oklahoma*

No anthropogenic contaminants detectable by our methods were identified in any water samples. One positively identified compound, 2-methyl-1(1,1-dimethyl) propanoic acid, which is a decomposition product of wood, was detected in the sediment samples. No compounds were detected above detection limits (<0.005µg/l) in water samples taken for benzene, toluene, ethylbenzene, and xylene (BTEX) analysis.

TABLE 4. Water(W) and Sediment (S) Analysis

Compound	Station 1(I)	2(I)	3(I)	4(R)
4,5-Dimethylnonane	W			
2-Methyl-2,2-dimethyl-1(1-methyl -ethyl)-1,3-propanediyl ester propanoic acid	W	W	W	
Diethyl phthalate	W	W		
α,ß-Dimethylbenzeneethanol	S			
1-Methylnaphthalene		S	S	
1,5-Dimethylnaphthalene		S		
2-Methylnaphthalene		S		
Anthracene		S		
9-Octadecen-1-ol		S		
Pyrene		S	S	
2,6,10-Trimethyldodecane		S	S	
Phenanthrene			S	
Benzo[a]pyrene			S	
Methylbenzene			S	
12-(Acetyloxy)-methyl ester 9-octadecenoate				S
(1-Methylethyl)benzene				S

(I = impacted, R = reference)

Fish Sampling Results

1. *Ardmore, Oklahoma*

A total of 365 fish were collected comprising six taxa (Table 5). The species diversity (Shannon Index) was highest for Station 3 (Table 6), the impacted station, with an H' of 1.525, an Hmax' of 2.584, and an evenness (H'/Hmax') of 0.59. The highest density (number of fish per unit area) was at Station 1, the

downstream station, with 211 individuals. Based on community similarity indices, Stations 1 and 2 and Stations 1 and 3 were the most similar with Percent of Similarities of 83.962% and 81.531% respectively and Morisita's Indices of 0.979 and 0.966 respectively (Table 7). Similarity indices also indicated that Stations 2 and 3 were less similar with a Percent Similarity of 65.493% and a Morisita's Index of 0.894.

TABLE 5. Fish Species and Numbers for Ardmore

Species	Station 1(D)	2(U)	3(I)	4(R)	5(R)	6(R)
Gambusia (*Gambusia affinis*)	178	9	93	0	0	0
Bluegill (*Lepomis macrochirus*)	15	0	24	1	0	6
Red Shiner (*Notropis lutrensis*)	17	0	14	0	0	0
Green Sunfish (*Lepomis cyanellus*)	0	0	3	1	0	0
Largemouth Bass (*Micropterus salmoides*)	0	0	2	0	0	0
Central Stoneroller (*Campostoma anomalum*)	2	0	6	0	0	2
Longear Sunfish (*Lepomis megalotus*)	0	0	0	0	0	1

(U = upstream, I = impacted, D = downstream, R = reference)

TABLE 6. Diversity Indices for Ardmore

	Station 1(D)	2(U)	3(I)
Total number of taxa	4	1	6
Total number of individuals	212	29	142
Shannon Diversity (H')	0.821	0	1.525
Hmax'	2.000	0	2.584
Evenness	0.4105	0	0.590

(D = downstream, U = upstream, I = impacted)

TABLE 7. Community Similarity Indices for Ardmore

	Station 1 & 2	2 & 3	1 & 3
Number of taxa present at both stations	1	6	6
Percent Similarity	83.962	65.493	81.531
Morisita's Index	0.979	0.894	0.966

2. *Cyril, Oklahoma*

A total of 134 fish were sampled comprising six taxa (Table 8). Several species of fish were observed at Station 1, Brown Pond. Due to the extremely soft bottom, brush, and extremely high conductivity, a single seining was done to evaluate the general population, and gill nets were set to sample the bullhead population.

Thirteen bullhead were taken from Station 1, and histologic analysis revealed that 23% of the bullheads had pigment deposits in the livers. This is

comparable to percentages exhibited by fish from other contaminated sites. Also, 31% had parasitic cysts. This is higher than percentages seen at some reference sites. One bullhead had a small vacuolated focus resembling a clear-cell focus that is considered a pre-neoplastic lesion in rats. Since the bullhead taken at Station 2 were juveniles, the livers were not taken for histopathology.

Station 3, the upstream station, had the highest diversity with an H' of 1.585 and an Hmax' of 1.585 (Table 9). Station 4 also had a high diversity with an H' of 1.362 and an Hmax' of 1.585. The highest density was seen at Station 4, the downstream station. Community similarity indices indicated that Stations 2 and 4 and Stations 3 and 4 had fairly equal similarities with Percent Similarities of 46.341 and 43.902 respectively and Morisita's Indices of 0.703 and 0.512 respectively (Table 10). Stations 2 & 3 were not similar.

TABLE 8. Fish Species and Numbers for Cyril

Species	Station 1(P)	2(I)	3(U)	4(D)	5(R)	6(R)	7(R)
Gambusia (Gambusia affinis)	25	10	0	19	0	0	0
Bluegill (Lepomis macrochirus)	15	0	6	18	0	0	0
Bullhead (Ameiurus melas)	17	2	0	0	0	0	0
Green Sunfish (Lepomis cyanellus)	3	0	6	0	2	0	0
Largemouth Bass (Micropterus salmoides)	5	0	0	4	0	0	0
White Crappie (Pomoxis annularis)	4	0	0	0	0	0	0

(P = pond, I = impacted, U = upstream, D = downstream, R = reference)

TABLE 9. Diversity Indices for Cyril

	Station 2(I)	3(U)	4(D)
Total number of taxa	2	2	3
Total number of individuals	12	12	41
Shannon Diversity (H')	1.0271	1.585	1.362
Hmax'	1.585	1.585	1.585
Evenness	0.648	1	0.8593

(I = impacted, U = upstream, D = downstream)

TABLE 10. Community Similarity Indices for Cyril

	Station 2 & 3	3 & 4	2 & 4
Number of taxa present at both stations	0	1	1
Percent Similarity	0.00	43.902	46.431
Morisita's Index	0.00	0.512	0.703

3. *Okmulgee, Oklahoma*

A total of 183 fish were captured in Okmulgee Creek comprising 13 taxa (Table 11). Histological examination revealed that 75% of the bullheads at Station 1 had normal livers compared to 40% at Station 2. Also, 20% from Station 2 had parasitic cysts and none from Station 1. Station 2 also had a high percentage, 60%, of the bullheads that had reactive/degenerative foci compared to none at Station 1. Bullhead from Station 3 were juveniles and livers were not taken. Station 1 had the highest diversity with an H' of 3.3058 and an Hmax' of 3.584 (Table 12). Station 3 had the highest density. Similarity indices indicated that Stations 2 and 3 are the most similar with a Percent Similarity of 40.302 and a Morisita's Index of 0.444 (Table 13).

TABLE 11. Fish Species and Numbers for Okmulgee

Species	Station 1(U)	2(I)	3(D)	4(R)	5(R)	6(R)
Green Sunfish (Lepomis cyanellus)	0	16	2	0	0	0
Channel Catfish (Ictalurus punctatus)	0	0	1	1	0	0
Slough Darter (Etheostoma gracile)	0	0	2	0	0	0
Gambusia (Gambusia affinis)	0	0	1	5	0	0
Bullhead (Ameiurus melas)	8	5	3	0	0	0
Largemouth Bass (Micropterus salmoides)	6	2	0	0	0	0
Carp (Cyprius carpio)	3	1	1	0	0	0
Bluegill (Lepomis macrochirus)	3	11	17	1	5	1
White Crappie (Pomoxis annularis)	2	1	1	0	0	0
Warmouth (Chaenobryttus gulosus)	0	23	7	0	0	0
Red Shiner (Notropis lutrensis)	0	3	21	1	1	0
Ghost Shiner (Notropis buchanani)	0	0	2	0	0	0
(Pimephales vigilax)	0	0	21	0	0	0
Gizzard Shad (Dorosoma cepedianum)	0	0	0	0	1	5

(U = upstream, I = impacted, D = downstream, R = reference)

TABLE 12. Diversity Indices for Okmulgee

Species	Station 1(U)	2(I)	3(D)
Total number of taxa	5	8	12
Total number of individuals	22	62	79
Shannon Diversity (H')	3.306	2.681	2.704
Hmax'	3.584	3.584	3.584

(U = upstream, I = impacted, D = downstream)

TABLE 13. Community Similarity for Okmulgee

	Station 1 & 2	1 & 3	2 & 3
Number of taxa present at both stations	5	4	7
Percent Similarity	28.152	19.965	40.302
Morisita's Index	0.293	0.227	0.444

4. *Arkansas River, Tulsa*

A total of 69 channel catfish were sampled. A general linear model variation of an analysis of variance was applied to the weights, lengths, and relative weights in order to compare the impacted stations and the control station. There was a significant difference in all three measurements (Table 14). The fish from the reference station were significantly heavier (reference = 482 g, impacted = 463 g, P = 0.0001), longer (reference = 1343 mm, impacted = 880 mm, P = 0.0356), and the relative weights were higher (reference = 102%, impacted = 81%, P = 0.0001) than fish collected at the reference station. There were no differences between the relative weights of the catfish comparing between age classes within each station (P = 0.1198). Morphological deformities were noted in the forms of clubbed, split,

and missing barbels. Twenty of the 53 fish from the impacted stations exhibited barbel deformities.

TABLE 14. Morphological Analysis of Channel Catfish

Dependent Variable	F Value	Probability > F
Length	4.61	0.0356
Weight	19.23	0.0001
Relative Weight	36.57	0.0001
Age (Relative Weight)	2.02	0.1198

Results of histopathological analysis are shown in Table 15. Liver sections from the impacted stations had some mottling and showed early signs of cellular alterations. There were significant differences between the impacted stations and the reference station in the numbers of fish with toxic changes. There were 15 fish from the impacted stations with toxic changes and none from the reference station ($P = 0.01$). The toxic changes were identified as cellular changes consistent with those observed in similar chemical exposures. There was also a significant difference in the number of fish containing parasitic cysts. There were 24 from the impacted stations and only 3 from the reference station ($P = 0.01$). A significant difference was also observed in the numbers of livers with cells exhibiting reactive/degenerative focus. There were two at the reference station and none at the impacted stations ($P = 0.008$).

TABLE 15. Results of Histopathologic Examination of Channel Catfish

Lesion Type	No. Lesions Observed (Percentage) Impacted	Reference
1	32 (60)	12 (75)
2	15 (28)	0 (0)
3	8 (15)	1 (6)
4	24 (45)	3 (18)
5	3 (6)	0 (0)
6	3 (6)	1 (6)
7	3 (6)	0 (0)
8	0 (0)	2 (13)

Description of Lesions
1) No visible lesions
2) Toxic change
3) Pigment deposits
4) Parasitic cysts
5) Focal vacuolated hepatocytes
6) Pericholangiolar fibrosis and other biliary lesions
7) Focal lymphocytic infiltration
8) Reactive/degenerative focus

5. *Arbuckle Lake, Murray County, Oklahoma*

A total of 374 adult and approximately 200 juvenile gizzard shad were captured. Forty-seven other individuals were captured comprising six taxa (Table 16). Lesions that were taken from gizzard shad were sent to Dr. William Hawkins of the Gulf Coast Research Laboratory and Dr. John Harshbarger, Director of the Registry of Tumors in Lower Vertebrates, Smithsonian Institute, for verification. They both concluded that the lesion was a neurofibroma. Based on our sampling of the Lake, there was a 16.73% occurrence of neurofibroma tumors in the adult gizzard shad population (Note: subsequent studies and sampling suggest close to

21-22% incidences). This was probably a very conservative estimate because it was based on lesions that could be seen with the unaided eye. The lesions were observed in adult fish with a mean length of 33.37 cm (Table 17). Thus, we estimated the fish to be 2 to 3 years in age. Among the 200 juveniles seined along shore and examined, no lesions were observed. Gizzard shad comprised 91% of fish captured as was expected based on our sampling methodology. Statistically significant differences were observed in the length, weight, and relative weights using a general linear models procedure in SAS, which is a modified analysis of variance for unbalanced data. The fish without lesions were shorter (342 mm vs. 351 mm) and weighed less (414 g vs. 434 g), but their relative weight was higher (111% vs. 107%) than that of fish with lesions.

TABLE 16. Fish Species and Numbers

Species	Numbers	Percent
*Gizzard Shad (Adult) (Dorosoma cepedianum)	374	91
Largemouth Bass (Micropterus salmoides)	8	2
White Crappie (Pomoxis annularis)	10	2
Carp (Cyprinus carpio)	12	2
Shortnose Gar (Lepisosteus oculatus)	4	1
White Bass (Morone chrysops)	12	2
Spotted Sucker (Minytrema melanops)	1	<1

* In addition, approximately 200 juvenile fish were seined along shore.

TABLE 17. Gizzard Shad Tumor Frequencies in Arbuckle Lake

08-06-91	49	2	28.9 - 33.4	4.08
08-07-91	56	13	26.2 - 34.4	23.21
12-05-91	11	1	27.5 - 30.5	9.09
04-24-92	80	5	30.0 - 40.5	6.25
08-21-92	69	7	27.0 - 30.0	10.14
09-12-92	11	3	33.5 - 36.5	27.27
01-06-93	107	31	31.0 - 39.5	28.97
03-27-93	270	67	29.6 - 39.7	24.81
Totals	**653**	**129**	**26.2 - 40.5**	***16.73**

*This is the mean percentage of the eight sampling trips.

SUMMARY AND CONCLUSIONS

1. *Ardmore, Oklahoma*

There were differences in the number and classes of compounds found in the water and sediments at the impacted, upstream, and downstream stations. There were about three times as many anthropogenic compounds present at the impacted and downstream stations compared to the upstream station. In addition, non-point sources are probably contributing to the contaminant load in Sand Creek. Immediately upstream from Station 2 is the old city dump. There are possibly leachates from run-off entering the creek at that point.

There were many more fish caught at the impacted and downstream stations compared to the upstream station. Similarly, these two stations had higher species diversities than the upstream station. There was also more water at these locations. The refinery (Station 3) and the wastewater treatment plant (WWTP) (Station 1) are probably contributing fairly significant amounts of water

to the stream. A report by the Oklahoma Water Resources Board (OWRB) (13) and one by Stanley Engineering (14) both showed that water flows increase below areas where effluents are being discharged. This could be providing a more varied environment for the fish; thus, densities and diversities would rise. However, the diversity did go down between the impacted station and the downstream station. The increased organic load from the WWTP could possibly be affecting the fish population at that point. Further sampling farther downstream would be needed to make a determination. Similar results have been reported near other wastewater treatment plants. The OWRB (13) reported that the WWTP at Cushing, Oklahoma, was causing an impact on the fish population of Cottonwood Creek, which receives the effluent. The species diversity was lowered below the WWTP and also lower than the reference creek.

There was a severe infestation of parasites on the Gambusia caught at Station 3. The parasites were myxozoans and were found in high numbers on most of the Gambusia caught at Station 3. They were not found at the other two stations. The increased pollution at Station 3 was probably a contributing factor for the parasite infestation. Kuperman (15) showed that parasites can be indicators of pollution.

Further sampling of the fish, water, and sediments is needed to make any definitive statements about the effects of the refinery on the fish population.

2. *Cyril, Oklahoma*

There were five times as many compounds at the impacted station compared to the two upstream stations and 13 times as many as the downstream station. Station 2 (impacted) was the only station with compounds in the water.

Although fish numbers were low, the upstream and downstream stations had higher diversities than the impacted station. Only 46% of the bullhead had no visible lesions in the liver. This was lower than the 60% observed in the channel catfish population from Tulsa. Thirty-one percent had parasitic cysts. This was lower than the impacted stations at Tulsa but higher than the reference station. Since no bullheads were collected at the upstream or downstream stations, no statistical analysis was performed. One bullhead did have a lesion consistent with pre-neoplastic lesions in rats (16).

Based on our results, there is an impact on the fish population. Further sampling of the bullhead population in Brown Pond would probably produce more lesions indicative of pre-neoplastic conditions.

3. *Okmulgee, Oklahoma*

There were differences in the number and classes of compounds found in the water and sediments at the impacted, upstream, and downstream stations. There were six times as many compounds present at the upstream station compared to the other two stations.

Compounds such as the phthalate esters and pyrenes are common to aquatic environments associated with this type of contamination sources. The phthalate esters are plasticizers in many plastics and are released into the environment as the plastics break down. The cyclic hydrocarbons are by-products formed in the refining of crude petroleum and during the incomplete combustion of fossil fuels. The fact that more compounds were identified upstream indicates that sources other than the refinery are contributing to the pollution in the creek. Station 2, the station at the refinery, was far from clean. The sediment was tar-

like and smelled like petroleum. The fact that only two compounds were positively identified there was due to the difficulty of separating complex chemical mixtures enough to obtain positive identification. Our chromatograms exhibited a typical "hydrocarbon hump" typical of the complex mixtures associated with oil refineries. If all compounds listed had been positively identified, there wouldhave been as many compounds at Station 2 as at Station 1. Common compounds such as pyrenes, anthracenes, and chrysenes were tentatively identified at Station 2.

Diversity indices and histological examination showed that Station 1, the upstream station, was in better shape biologically than Station 2. The diversity was highest, and there was a higher percentage of fish with normal livers at Station 1. Station 2 had a lower diversity than both of the other stations. Also, 60% of the fish at Station 2 had livers with reactive/degenerative focus.

Based on our results, there are anthropogenic contaminants in Okmulgee Creek. There are compounds present that are in the classes of compounds that have been shown to cause morphological deformations and neoplastic lesions in fish. We also can say that there are differences in the community parameters of the fish. These may be caused by the contamination from the oil refinery or by other non-point sources along the creek. Further sampling of the fish, water, and sediments is needed to make any definitive statements about the area.

4. *Arkansas River, Tulsa, Oklahoma*

There were differences in the number and classes of compounds found in the water and sediments at the impacted stations and the reference station. There were almost five times as many compounds present at the impacted stations than the reference station.

Significant differences in weight, length, standard length, and relative weights were apparent between the impact and reference stations (17). This observation, coupled with the observed differences in water quality, suggests a possible cause and effect relationship. Results of histopathological examination revealed differences in liver conditions between the two populations with some statistically significant differences. The fish from impacted stations had fifteen occurrences of toxic changes. This was 28% of the fish that we sampled. No fish from the reference station exhibited toxic changes in the liver. Also, as is common with polluted locations, a significantly higher number of fish at the impacted stations had parasitic cysts in the liver: 45% at impacted stations and 18% at the reference station. In general parasitic infections are not lethal to the fish but in high densities can be very harmful and ultimately compromise reproductive success.

Contamination from the oil refineries and/or other point and non-point sources entering the river through the stormwater drains may be impacting the channel catfish population. The significant differences in the morphometric measurements along with those of histologic importance suggest that this is a problem with potentially enormous impacts. The relative weight, which is an indicator of overall animal health, was significantly lower in fish collected from the impacted stations than that of those collected at the reference station. This could be caused by direct and/or indirect effects. An indirect effect would be that of a reduced forage base that could be investigated by extensive sampling of the river. With a reduced forage base, the catfish population would possibly have a nutritional imbalance in their diet. A direct effect would be that of the

contaminants affecting the sensory and metabolic pathways of the catfish. The fish could also be suffering from narcotization and thus be unable to effectively catch prey. The fish could also have reduced metabolism and thus not be getting the full nutritional value from the prey that they catch. Detailed analysis of catfish tissues for appropriate contaminants or their metabolites would be necessary to verify this hypothesis.

An equally important effect, mentioned previously, is that of reproduction. The average age of fish from the reference station was 4.3 years, and those from the impacted stations averaged 3.7 years. The average age of the population from the impacted stations may be decreasing because fewer fish are reaching sexual maturity or are not remaining at a sexually mature age long enough to reproduce for one or more seasons [Note: a larger sample size is needed before this hypothesis can be supported].

From these preliminary studies and the data presented herein, we conclude that significant differences exist between reference and impacted stations in terms of water and sediment quality. In particular, the presence of anthropogenic contaminants, especially known carcinogens (e.g., benzo[a]pyrene) are cause for concern. Moreover, we have documented significant differences in the channel catfish populations residing at the two areas in terms of histopathology (liver) and morphometric analyses. To date, we have not observed any cancerous lesions, nonetheless, the presence of significant toxic liver changes among catfish collected from the impacted sites suggests a potential for this to occur.

5. *Arbuckle Lake, Murray County, Oklahoma*

This is the first epizootic of neurofibroma ever reported in a freshwater species. Sampling of Arbuckle Lake revealed no obvious contamination sources. Since we did not analyze for viruses, heavy metals, or radiation, and no definitive reports are available on this data, we cannot rule them out as possible causes of the lesions. These are currently being investigated.

Similar to reports of neurofibromatosis in the damselfish (18,19), the gizzard shad are in apparently pristine water. A similar type of lesion has been previously described in the bicolor damselfish. Lesion prevalence in the damselfish has been reported to be 0.1-23% (18). The population of gizzard shad that we studied exhibited a 17% occurrence. Based on our sampling, there are significant numbers of fish exhibiting neurofibromas, and the percentages are comparable to the occurrence of neurofibromatosis in the damselfish. While gizzard shad are consistently caught at this location exhibiting neurofibromas, this lesion has not been reported in any other Oklahoma species (Jimmie Pigg, personal communication). Results of sampling in four locations at several different times showed that the gizzard shad population was highly concentrated in the area of the lake that we were sampling in. It is unknown why this was so, but it correlates to data reported by Schmale and colleagues (18) that the lesions they observed were more prevalent in concentrated populations.

Fish with lesions were longer and weighed more, but their relative weight was less (20). This can be explained by making the assumption that fish exhibiting lesions were older than fish without; thus, they were longer and heavier. However, the fact that they were affected with the lesion possibly reduced

their overall fitness and thus reduced their relative weight. The relative weight would be reduced in fish not utilizing their biomass intake optimally (21). The etiology and transmissibility of the neurofibroma in this population of gizzard shad are currently being studied.

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BIBLIOGRAPHY

1. Toxics Monitoring Survey of Oklahoma Reservoirs. Oklahoma State Department of Health. T1985 - 1989. 212 pp.

2. Malins, D. C. (ed.) Toxic Chemical and Aquatic Life: Research and Management Elsevier Science Publishers B.V., Amsterdam 1988.

3. Ostrander, G.K., and D. C. Malins. Chronic fish toxicity: the complementary nature of field and laboratory studies with reference to Puget Sound, USA. In Proceedings of the V International Congress of Toxicology: Basic Science in Toxicology. 174-183, 1990.

4. Rock, B. J. Oil and Gas Journal Data Book. Penn Well Publishing Company. Tulsa, Oklahoma. 1991.

5. Kinney, G. T. (ed.) U. S. Refineries: Annual Production Report. Oil and Gas Journal. 80:142, 1982.

6. Simpson, E. H. Measurement of diversity. Nature. 163:688, 1949.

7. Krebs, C. J. Ecology: The Experimental Analysis of Distribution and Abundance. Harper and Row, New York. 1972.

8. Brower, J. E., and J. H. Zar. Field and Laboratory Methods for General Ecology. Wm. C. Brown Company Publishers. U.S.A. 1977.

9. Morisita, M. Measuring of interspecific association and similarity between communities. Mem. Fac. Sci. Kyushu Univ., Ser. E (Biol) 3:65-80, 1959.

10. Heller, S. R., and G. W. Milne. NBS Mass Spectral Data Base. Supplement #1. U. S. Dept. of Commerce/Natl. Bureau of Standards. 1980.

11. Malins, D. C., B. B. McCain, D. W. Brown, A. K. Sparks, and H. O. Hodgins. Chemical Contaminants and Biological Abnormalities in Central and Southern Puget Sound. NOAA Technical Memorandum. OMPA-2 1980.

12. Fabacher, D. L., C. J. Schmitt, J. M. Besser, and M. J. Mac. Chemical characterization and mutagenic properties of polycyclic aromatic compounds in sediment from tributaries of the Great Lakes. Environmental Toxicology and Chemistry. 7:529-543, 1988.

13. Use Attainability Analysis of Cottonwood Creek. Oklahoma Water Resources Board. 1989.

14. Stanley Engineering. Oklahoma Refining Company: Environmental and Hydrological Assessment. Vol. 1. Oklahoma City, Oklahoma, 1985.

15. Kuperman, B. I. Parasites as bioindicators of the pollution of water bodies. Parazitologiya. 26:479, 1992.

16. Farber, E., and R. Cameron. The sequential analysis of cancer development. Adv. Cancer Res. 31:297-321, 1980.

17. Ostrander, G.K. and Kuehn, R.L. Evaluation of water, sediments, morphometircs, and liver histopathology of channel catfish collected from impacted and reference sites along the Arkansas River, Tulsa, OK. U.S. Fish and Wildlife Service Technical Report. 1993.

18. Schmale, M. C., G. Hensley, and L. R. Udey. Neurofibromatosis, von Recklinghausen's disease, multiple schwannomas, malignant schwannomas. Multiple schwannomas in the bicolor damselfish, Pomacentrus partitus (Pisces, Pomacentridae). Am. J. of Path. 121:238-241, 1983.

19. Schmale, M. C., and G. T. Hensley. Transmissibility of a neurofibromatosis-like disease in bicolor damselfish. Cancer Res. 48: 3828-3833, 1988.

20. Kuehn, R.L. Anthropogenic Contaminants and Fish Health at Five Sites in Oklahoma. M.S. Thesis. Stillwater: Oklahoma State University. July 1993.

21. Wege, G. J., and R. O. Anderson. Relative weight (Wr): a new index of condition for largemouth bass. Pages 79-91 in G. D. Novinger and J. G. Dillard, editors. New approaches to the management of small impoundments. American Fisheries Society, North Central Division, Special Publication 5, Bethesda, Maryland, 1978.