

**Fish and Macroinvertebrate
Study of
Fish Creek
DRAFT
1997**

Steuben and Dekalb Counties, Indiana
Williams County, Ohio

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Introduction/ Methods

This report is an update to the *Fish and Macroinvertebrate Study of Fish Creek 1994*, Ohio EPA (1995) and *Addendum to Fish and Macroinvertebrate Study of Fish Creek 1994*, Ohio EPA (1996a). Ohio EPA, as one of the Natural Resource Damage Assessment (NRDA) trustees for Fish Creek, collected in-stream biological data to assess the condition of the stream in 1994, 1995, and 1997. Ohio EPA had previously collected biological data in 1991 and 1992 and was conducting a routine basin survey in 1993, when the diesel fuel spill occurred. A detailed discussion of the previous survey results through 1995 can be found in Ohio EPA (1993a; 1993b; 1995; 1996a). Fish and macroinvertebrate communities were sampled during the summer and fall of 1997 at six locations in Fish Creek from river mile (RM) 21.6 to the mouth (Table 1, Figure 1). Sampling was conducted to continue the assessment of fish and macroinvertebrate community conditions following a rupture in a pipeline which spilled approximately 30,000 gallons of #2 diesel fuel into Fish Creek at RM 7.55 on September 15, 1993. Oil collection booms were placed in Fish Creek shortly after the spill, and reportedly the spill was confined before entering the St. Joseph River. Fish collections were made at each site in August and September 1997 using pulsed DC electrofishing gear, with sampling distance varying between 190 and 220 meter zones. Macroinvertebrate collections were made at each site using modified Hester-Dendy multiple-plate artificial substrate samplers colonized from August 11 - September 15, 1997. At the time of sample retrieval, a qualitative sample of the macroinvertebrate community was collected from all available natural substrates in the near vicinity of the sampling site. The macroinvertebrate quantitative artificial substrate and qualitative natural substrate samples and fish samples were collected by Ohio EPA and USFWS biologists; laboratory work, data processing and data analysis were conducted by Ohio EPA. Fish and macroinvertebrate field work, laboratory, data processing and data analysis methods and procedures conducted by Ohio EPA were consistent with those specified in Ohio EPA manuals (1987, 1989a, 1989b).

Stream sediment and surface water samples were collected from each biological station in Fish Creek. Fine grained sediment samples were collected in the upper six inches of bottom material at each location using decontaminated stainless steel scoop samplers (Ohio EPA 1996b). Collected sediment was homogenized and then placed into decontaminated clear glass jars with teflon lined lids, placed on ice (to maintain 4°C) and shipped to an Ohio EPA contract lab. Sediment data is reported on a dry weight basis. Sediment evaluations were conducted using consensus-based sediment guidelines (MacDonald *et al.* 2000), reference conditions and published literature. Surface water grab samples were collected twice from each sampling location. Chemical parameters measured in surface waters included metals, nutrients, solids, chloride, sulfate, oxygen demand, acidity, alkalinity, and coliforms. Water samples were collected directly into sample containers and appropriate preservatives added. Water samples were delivered to an Ohio EPA contract lab for analysis.

Common carp were collected from five sites for biomarker processing during the 1997 community assessment sampling. Fish were kept in a floating livewell until biomarker tissue samples could be taken. Fish were anesthetized in MS222 and length and weight measured. Fish health/ condition was assessed using procedures described in Goede (1988). Blood was drawn from the caudal vein through a 21 gauge needle into heparin treated 3 ml blood drawing tubes. Whole blood was centrifuged on-site and the plasma removed (flash frozen at -100EC in a liquid nitrogen dry shipper). The liver was excised, wrapped in aluminum foil and frozen in a liquid nitrogen dry shipper. Bile was removed, placed in amber microcentrifuge tubes and frozen in a liquid nitrogen dry shipper. Tissue samples were transported to the U.S. EPA in Cincinnati for laboratory analysis. Specific biomarker analyses included ethoxyresorufin-O-deethylase (EROD), blood urea nitrogen, and bile metabolites. Each bile sample was diluted with distilled/deionized water and measured by fixed fluorescence at four excitation/emission wavelength pairs

according to Lin et al. (1996). Although more than one compound is known to fluoresce under these conditions, some compounds give a greater response. The metabolites are referred to by one of their most sensitive respondents: pyrenol-type at 340/380 nm, benzo(a)pyrenol-type at 380/430 nm, phenanthrol-type at 256/380 nm and naphthol-type at 290/335 nm. Microsomes for measuring EROD were prepared from liver tissue according to Lin et al. (1989). EROD activity was measured fluorometrically according to Pohl and Fouts (1980) and modified Lin et al. (1989).

Summary/ Conclusions

From August to September, 1997 Ohio EPA Division of Surface Water staff, at the request of the Division of Emergency and Remedial Response, conducted biological community, sediment, and surface water sampling of Fish Creek upstream and downstream from a diesel fuel spill which occurred September 15, 1993. In addition, biomarker samples were taken from common carp at five sites in Fish Creek. The results of these sampling events are summarized below. The portion of Fish Creek in Indiana was evaluated using the Ohio EPA Exceptional Warmwater (EWH) biological criteria.

A total of 22.1 miles of Fish Creek was assessed by the Ohio EPA in 1997. Based on the performance of the biological communities, 18.0 miles of Fish Creek were in partial attainment of the Exceptional Warmwater Habitat/Warmwater Habitat aquatic life uses and 4.1 miles were in non-attainment of the EWH use (Table 2). The partial and non -attainment was due primarily to the failure of the fish community to meet biological criteria. The lower IBI scores in Fish Creek in 1997, when compared to previous years, appears to be at least partly associated with a heavy silt layer covering the bottom substrates. The heavy silt layer pervasive in 1997 was the most extensive observed during the last three sampling years (1994, 1995 and 1997). As in past years, macroinvertebrate sampling documented exceptional conditions at nearly all locations, excluding RM 5.4. There was no obvious cause for the decline exhibited by the macroinvertebrate community at RM 5.4. Fish communities were indicative of marginally good to good conditions, excluding RM 0.3, where a fair quality fish community occurred. Although a number of pollution sensitive fish species characteristic of exceptional habitat quality were recorded in Fish Creek, their numbers were overshadowed by pollution tolerant species.

Based on the limited number of water samples collected in this study, chemical water quality of Fish Creek was considered marginally good, with only fecal coliform and total phosphorus exceeding Ohio's WQS criteria and guidelines. Fecal coliform concentrations measured at all locations on September 13 (elevated stream flow) exceeded Ohio's WQS criterion for the Primary Contact Recreation use. Elevated total phosphorus levels at RM 0.3 suggest that excessive nutrient enrichment is occurring in the lower section of the stream. All other chemical parameters were either within acceptable water quality criteria levels, were reported by the laboratory as not detected, or were at or below ecoregional reference conditions.

Very low levels of benzo(a)pyrene, benzo(b)fluoranthene, benzo(g,h,i)perylene, benzo(a)anthracene, benzo(k)fluoranthene, fluoranthene, and pyrene were documented in the sediment at RM 5.4 and RM 0.3. These same parameters were not detected upstream from the diesel fuel spill site. Although PAH compounds were detected in Fish Creek downstream from the diesel fuel spill site, concentrations were

below *Threshold Effect Concentrations* - a level below which harmful effects to aquatic biota are unlikely to occur. The moderately strong diesel fuel odor noted in bottom sediments from RM 5.4 during 1994 was not evident during 1997.

Biomarker results (EROD and bile metabolites) from Fish Creek in 1997 showed no significant difference between sampling locations. All fish tested had PAH type bile metabolites and EROD levels below reference levels, suggesting little or no exposure to organic contaminants.

Table 1. Fish and macroinvertebrate sampling locations in Fish Creek, 1997.

Stream/ River Mile	Latitude	Longitude	Landmark	County	USGS 7.5 min. Quad. Map
<i>Fish Creek</i>					
21.6/21.7	41E34'09"	84E49'42"	TR 850E, Indiana	Steuben	Edon, Ind.-Ohio
14.3	41E30'59"	84E52'11"	CR 4A, Indiana	Dekalb	Edon, Ind-Ohio
8.3	41E28'27"	84E49'36"	CR 16, Indiana	Dekalb	Butler East, IN-OH
7.5	41E27'55"	84E49'34"	RR track, Indiana	Dekalb	Butler East, IN-OH
5.4	41E27'58"	84E48'05"	TR 171, Ohio	Williams	Butler East, IN-OH
0.3	41E27'48"	84E44'51"	SR 49, Ohio	Williams	Edgerton, Ohio

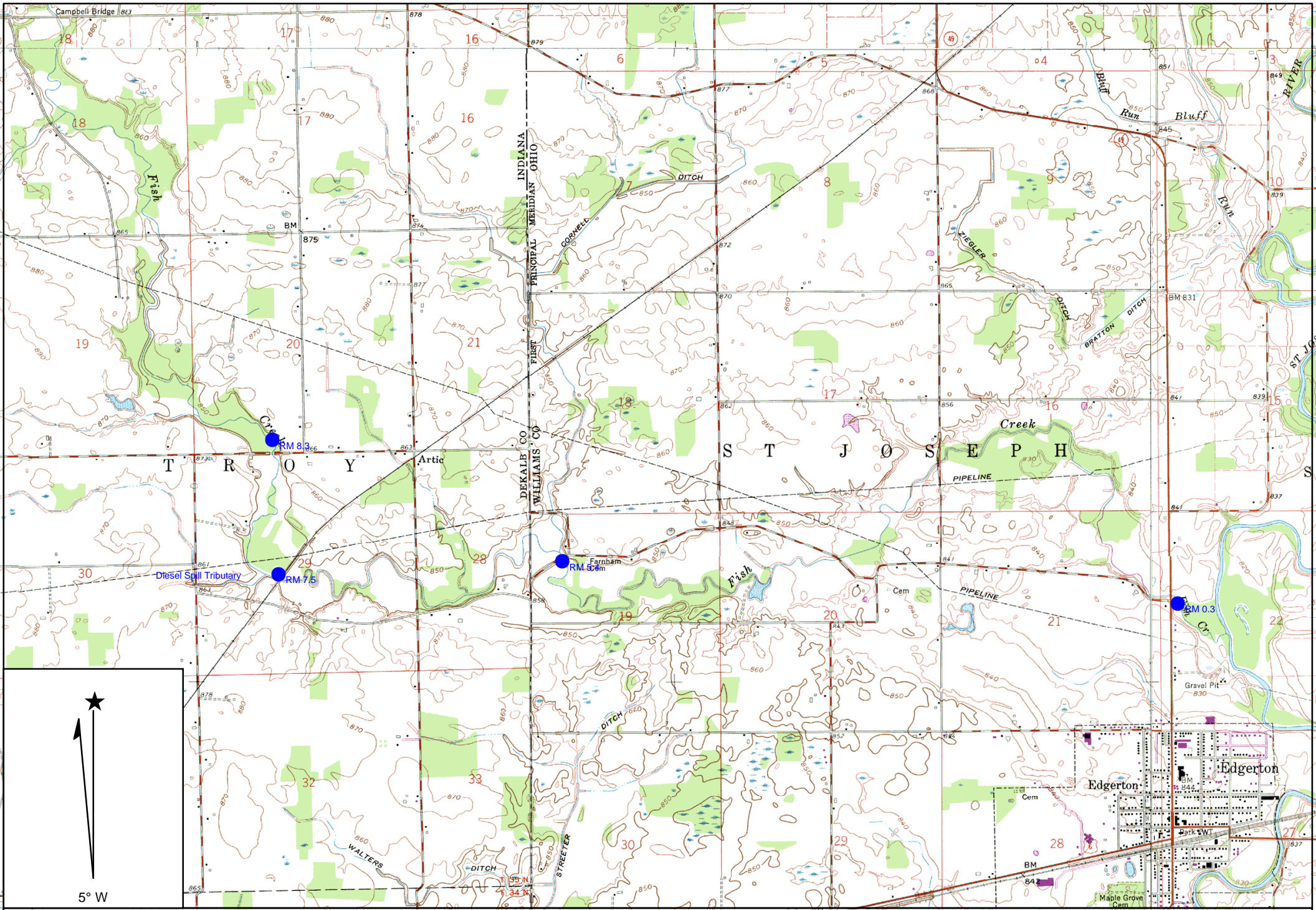
Table 2. Aquatic life use attainment status for Fish Creek based upon sampling conducted from August to September, 1997. Attainment status is based on biocriteria for the Eastern Corn Belt Plains ecoregion of Ohio (OAC Chapter 3745-1-07, Table 7-15).

RIVER MILE Fish/Invert.	IBI	Modified Iwb	ICI	QHEI	Attainment Status	Comment
Fish Creek-1997		<i>Eastern Corn Belt Plains Ecoregion - EWH Use Designation</i>				
21.6/ 21.7	36*	8.5*	54	75.0	PARTIAL	Background/small stream
14.3/ 14.3	39*	8.1*	52	78.0	PARTIAL	Downstream Hamilton
8.3/ 8.3	43*	8.2*	46	76.0	PARTIAL	Upstream diesel spill
7.5/ 7.5	39*	8.3*	50	73.5	PARTIAL	Diesel spill area - near field
5.4/ 5.4	42*	8.7*	40*	75.0	NON	Diesel spill area - far field
<i>Eastern Corn Belt Plains Ecoregion - WWH Use Designation</i>						
0.3/ 0.3	30*	7.0*	50	69.0	PARTIAL	Downstream recovery

Ecoregion Biocriteria: Eastern Corn Belt Plains (ECBP)

<u>INDEX</u>	<u>WWH</u>	<u>EWI</u>	<u>MWH^a</u>
IBI - Wading	40	50	24
Mod. Iwb - Wading	8.3	9.4	6.2
ICI	36	46	22

-
- * - Significant departure from ecoregion biocriterion (>4 IBI or ICI units; >0.5 MIwb units).
 - ^{ns} - Nonsignificant departure from ecoregion biocriterion (≤4 IBI or ICI units; ≤0.5 MIwb units).
 - ^a - Modified Warmwater Habitat for channel modified areas.



Name: BUTLER EAST
 Date: 6/13/2002
 Scale: 1 inch equals 2857 feet

Location: 041° 28' 23.4" N 084° 47' 34.8" W
 Caption: Figure 1. Map of the Fish Creek study area showing Ohio EPA sampling locations (by river mile), 1997. page 9

Surface Water Chemistry

Surface water chemical analyses were conducted on grab samples collected from Fish Creek between RM 21.6 and RM 0.3 during 1997. Two samples were collected from each location; once in August and once in September. Water samples were tested for eight metals, acidity, biochemical oxygen demand, chloride, coliforms, nitrogen compounds, phosphorus, alkalinity, sulfate, total suspended solids and total dissolved solids. Results of these tests are reported in Table 3 and summarized below.

Many of the water quality parameters measured in Fish Creek during August and September, 1997, were within acceptable levels. Ammonia-N, total dissolved solids, arsenic, chromium, lead, mercury, and selenium were below Ohio Exceptional Warmwater Habitat water quality criteria. Alkalinity, chloride, nitrate-N, and sulfate concentrations were low based on values below the 90th percentile concentration for reference conditions in the Eastern Corn Belt Plain Ecoregion. Acidity and barium values were near or below the parameter laboratory detection limit. Although cadmium and silver were not detected in any water quality samples, the laboratory detection limits were high for these two chemicals.

Summer base flow conditions occurred in Fish Creek during the August 13 water quality sampling event. However, during the September 17 water chemistry sampling event, elevated stream flows and excessive suspended sediment existed in Fish Creek and its tributaries. These conditions resulted from heavy rainfall during the previous night and early morning. Chemical parameters with increased concentrations during the elevated and turbid stream flow of September 13 included biochemical oxygen demand, total Kjeldahl nitrogen, total suspended solids, lead, fecal coliform and total coliform.

Fecal coliform concentrations measured at all six locations on September 13 exceeded Ohio's WQS criterion for the Primary Contact Recreation use. In addition, one total phosphorus sample collected at RM 0.3 exceeded the Ohio WQS guideline of 1.0 mg/l. Of the six locations sampled in Fish Creek, RM 0.3 had the highest levels of total phosphorus (3.1 and 1.0 mg/l) which suggested that excessive nutrient enrichment occurs in the lower section of the stream.

Overall water quality of the six surface water samples was marginally good, with only fecal coliform and total phosphorus exceeding Ohio's WQS criteria and guidelines. All other chemical parameters were either within acceptable water quality criteria levels, were reported by the laboratory as not detected, or were at or below ecoregional reference conditions.

Table 3. Results of analyses of grab water samples collected by Ohio EPA from Fish Creek during August 13 and September 17, 1997. ND = not detected at or above lab detection limit.

Parameter (Units)(Detection Limit)	Fish Creek - Surface Water					
	RM 21.6	RM 14.3	RM 8.3	RM 7.5	RM 5.4	RM 0.3
Acidity (mg/l)(10)	ND ND	ND ND	ND ND	ND ND	ND ND	ND ND
Alkalinity-Total (mg/l)(5)	300 220	280 170	270 140	280 87	290 150	290 220
BOD ₅ (mg/l)(2)	ND 3	ND 5	ND 4	ND 4	ND 4	ND 2
Chloride (mg/l)(1)	31 19	30 15	30 12	31 10	33 15	30 16
Ammonia-N (mg/l)(0.2)	ND 0.4	ND ND	ND ND	ND ND	ND ND	ND ND
Nitrate/Nitrite-N (mg/l)(0.1)	0.3 1.3	0.6 0.5	0.8 0.7	0.7 0.7	0.7 0.8	0.6 0.7
Nitrite-N (mg/l)(0.1)	ND ND	ND ND	ND 0.1	ND 0.2	ND ND	ND ND
TKN (mg/l)(1)	ND 2	ND 7	ND 6	ND 3	ND ND	ND 3
Phosphorus-T (mg/l)(0.1)	ND 0.3	0.2 0.3	ND 0.3	0.8 0.3	0.6 0.4	1.0 3.1‡
Dissolved Solids-T (mg/l)(10)	450 280	420 210	390 160	400 220	390 180	430 290
Suspended Solids-T (mg/l)(4)	18 80	17 85	13 320	21 240	21 200	33 120
Sulfate (mg/l)(55)	68 44	55 33	49 22	47 41	43 50	53 83
Fecal Coliform (#/100 ml)(10)	120 25,000*	110 4,000*	ND 12,000*	ND 12,500*	ND 7,500*	ND 3,000*
Total Coliform (#/100 ml)(10)	140 1,000	140 10,000	150 20,000	170 ND	130 1,000	130 2,000

Table 3. Continued.

Parameter (Units:Detection Limit)	Fish Creek - Surface Water					
	RM 21.6	RM 14.3	RM 8.3	RM 7.5	RM 5.4	RM 0.3
Arsenic-T (ug/l)(10)	ND ND	ND ND	ND ND	ND ND	ND ND	ND ND
Barium-T (ug/l)(200)	ND ND	ND ND	ND ND	ND ND	ND ND	ND ND
Cadmium-T (ug/l)(5)	ND ND	ND ND	ND ND	ND ND	ND ND	ND ND
Chromium-T (ug/l)(10)	ND ND	ND ND	ND 12	ND 16	ND ND	11 ND
Lead-T (ug/l)(3)	ND 6	ND 5.9	ND 7.5	ND 13	ND 11	ND 5.4
Mercury-T (ug/l)(0.2)	ND ND	ND ND	ND ND	ND ND	ND ND	ND ND
Selenium-T (ug/l)(5)	ND ND	ND ND	ND ND	ND ND	ND ND	ND ND
Silver-T (ug/l)(10)	ND ND	ND ND	ND ND	ND ND	ND ND	ND ND
pH -Field (S.U.)	8.4	8.3	8.3	8.2	8.3	8.2
Conductivity -Field (umhos/cm)	610	575	575	575	575	590
Temperature (°C)	19.2	19.4	19.7	19.4	19.5	19.2

‡ Exceeds Ohio WQS guidelines of daily average of 1.0 mg/l.

* Exceeds Ohio Primary Contact Recreational water quality criteria.

Sediment Quality

Sediment samples were collected at six locations in Fish Creek during August 1997. All sampling locations are indicated by river mile in Figure 1. Samples were analyzed for RCRA metals, polycyclic aromatic hydrocarbons (PAHs), and total recoverable petroleum hydrocarbons (TRPH). Specific chemical parameters tested and results are listed in Table 4.

Sediment samples were evaluated in part using consensus-based sediment guidelines (MacDonald *et al.* 2000). The guidelines define two levels of ecotoxic effects - threshold effect concentration (TEC) and probable effect concentration (PEC). The TEC is a level in sediment below which harmful effects to benthic organisms are unlikely to be observed and the PEC indicates a level above which harmful effects are likely to be observed. Based on the guidelines noted above, none of the Fish Creek sediment samples exceeded the TEC or PEC for numerous metals and polycyclic aromatic hydrocarbon (PAH) compounds (Table 4). The guidelines detailed in MacDonald *et al.* (2000) do not include evaluations of several PAHs and metals, and most non-PAH semivolatile organic compounds.

Very low levels of benzo(a)pyrene, benzo(b)fluoranthene, benzo(g,h,i)perylene, benzo(a)anthracene, benzo(k)fluoranthene, fluoranthene, and pyrene were documented in the sediment at RM 5.4 and RM 0.3. These same parameters were not detected upstream from the diesel fuel spill site. Although PAH compounds were detected in Fish Creek downstream from the diesel fuel spill site, concentrations were below *Threshold Effect Concentrations*. The moderately strong diesel fuel odor noted in bottom sediments from RM 5.4 during 1994 was not evident during 1997.

Comparison of Ohio EPA sediment PAH data from 1997 and results reported by the USFWS and Indiana Department of Environmental Management from September and November 1993 were not possible due to the differences in lab detection levels between the two data sets. Nevertheless, samples from both years reported low levels of PAH compounds at sites located upstream and downstream from the diesel fuel spill site.

Table 4. Chemical compounds detected in sediment samples collected by Ohio EPA from Fish Creek on August 13, 1997. Measurements in **bold** exceed the TEC as detailed in MacDonald *et al.* 2000. Parameters exceeding the PEC are indicated by underlined **bold** numbers. Parameters in *italics* do not have review guidelines established in MacDonald *et al.* 2000.

Parameter	Fish Creek Sediment (River Mile)					
	21.6	14.3	8.3	7.5	5.4	0.3
<i>Metals - Total (mg/kg)</i>						
Arsenic	2.9	3.0	4.4	3.4	3.5	3.4
Barium	39.5	32.5	52.5	35.8	24.3	24.9
Cadmium	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Chromium	5.7	6.2	10.1	6.3	6.8	4.9
Lead	5.1	4.1	5.9	4.9	4.2	4.3
Mercury	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Selenium	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Silver	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
<i>Polycyclic Aromatic Hydrocarbons (ug/kg)</i>						
Acenaphthene	<200	<200	<200	<200	<200	<200
Acenaphthylene	<200	<200	<200	<200	<200	<200
Anthracene	<200	<200	<200	<200	<200	<200
Benzo(a)anthracene	<6.7	<6.7	<6.7	<6.7	8.0PF	17PF
Benzo(a)pyrene	<6.7	<6.7	<6.7	<6.7	16	19
Benzo(b)fluoranthene	<6.7	<6.7	<6.7	<6.7	16	17
Benzo(g,h,i)perylene	<6.7	<6.7	<6.7	<6.7	11	10
Benzo(k)fluoranthene	<6.7	<6.7	<6.7	<6.7	<6.7	9.2
Chrysene	<20	<20	<20	<20	<20	<20
Dibenzo(a,h)anthracene	<6.7	<6.7	<6.7	<6.7	<6.7	7.6
Fluoranthene	<6.7	<6.7	<6.7	<6.7	15PF	43
Fluorene	<200	<200	<200	<200	<200	<200
Indeno(1,2,3-cd)pyrene	<6.7	<6.7	<6.7	<6.7	<6.7	<6.7
1-Methylnaphthalene	<200	<200	<200	<200	<200	<200
2-Methylnaphthalene	<200	<200	<200	<200	<200	<200
Naphthalene	<200	<200	<200	<200	<200	<200
Phenanthrene	<200	<200	<200	<200	<200	<200
Pyrene	<6.7	<6.7	<6.7	<6.7	19	26
<i>Total Recoverable</i>						
Petroleum Hydrocarbons (mg/kg)	10	<10	<10	<10	<10	<10
Total Organic Carbon (mg/kg)	>14,800	14,600	>15,500	10,000	6,160	8,590
Percent Solids	65.1	50.8	35.6	60.4	74.1	66.3

PF The percent difference between the original and confirmation analyses is greater than 50%.

Macroinvertebrate Community

Macroinvertebrate communities were sampled during August- September 1997 at six locations in Fish Creek from RM 21.7 to RM 0.3 (Table 1). Summarized results from the 1997 sampling as well as previous data from 1995, 1994, 1993, and 1992 are compiled in Table 5. ICI metrics with scores are in Appendix Table 1 and the raw data are listed in Appendix Table 2. Figure 2 displays ICI scores by river mile for the 1997- 1992 data. A discussion of the 1993 data is provided in Ohio EPA (1993a). The 1993 data, including a discussion of impacts from the September 15, 1993 diesel fuel spill, is provided in Ohio EPA (1993b). Sampling in 1994 to monitor biological recovery following the spill is discussed in Ohio EPA (1995). Monitoring data from 1995 is summarized in Ohio EPA (1996). Sampling locations in Indiana (RM 21.6- RM 7.5) and in Ohio at RM 5.4 were evaluated using Ohio's Exceptional Warmwater Habitat (EWH) ICI biocriteria. The site at RM 0.3 was evaluated using the Warmwater Habitat (WWH) ICI biocriteria established for the Eastern Corn Belt Plains ecoregion.

The macroinvertebrate communities at the four sampling sites in Indiana (RMs 21.7, 14.3, 8.3, and 7.5), with ICI scores of 54, 52, 46, and 50, respectively, reflected high quality communities achieving the EWH use. The macroinvertebrate community from the Ohio site at RM 5.4 had an ICI of 40 indicating non-attainment of the EWH use. The macroinvertebrate community from this site had reduced mayfly, caddisfly, and total taxa diversity and reduced mayfly abundance when compared to both upstream and downstream sites. There was no obvious cause for the decline exhibited by the macroinvertebrate community at RM 5.4. Downstream, at the RM 0.3 site, the macroinvertebrate community, with an ICI of 50, reflected attainment of not only the designated WWH use but the EWH use as well.

Table 5. Summary of macroinvertebrate data collected from artificial substrates (quantitative sampling) and natural substrates (qualitative sampling) in Fish Creek, 1992 - 1997. Fish Creek from RM 5.6 to RM 2.4 has an EWH aquatic life use designation and WWH from RM 2.4 to the mouth in the Ohio Water Quality Standards. Sampling locations in Indiana (RM 21.6 - RM 7.5) and in Ohio at RM 5.4 were evaluated using Ohio's Exceptional Warmwater Habitat biocriterion. The site at RM 0.3 was evaluated using the ecoregional Warmwater Habitat biocriterion.

Stream/ River Mile	Relative Density	Total Taxa	Quantitative Evaluation			ICI	Evaluation
			Quantitative Taxa	Qualitative Taxa	Qualitative EPT ^a		
<i>Fish Creek - 1997</i>							
21.7	273	69	52	40	15	54	Exceptional
14.3	157	62	46	38	14	52	Exceptional
8.3	194	55	44	27	13	46	Exceptional
7.5	484	50	40	26	11	50	Exceptional
5.4	325	47	34	32	13	40*	Good
0.3	402	54	44	29	14	50	Exceptional
<i>Fish Creek - 1995</i>							
21.7	451	74	51	50	20	54	Exceptional
14.3	758	62	46	40	16	54	Exceptional
8.3	716	59	43	38	15	48	Exceptional
7.5	860	82	47	56	14	56	Exceptional
5.4	688	72	44	54	12	50	Exceptional
<i>Fish Creek - 1994</i>							
21.7	568	58	38	32	7	40*	Good
14.3	801	48	36	25	8	48	Exceptional
8.3	1260	48	42	22	7	48	Exceptional
6.5	1701	65	41	45	9	52	Exceptional
5.4	774	73	44	43	6	38*	Good
0.3	842	72	46	42	12	50	Exceptional
<i>Fish Creek - 1993</i>							
21.7	658	87	46	69	20	56	Exceptional
17.1	290	65	43	44	14	52	Exceptional
13.8	417	69	47	37	11	44 ^{ns}	Very Good
9.9	761	70	52	39	14	46	Exceptional
5.4	155	45	32	24	7	20*	Fair
0.3	118	60	53	23	8	36	Good
<i>Fish Creek - 1992</i>							
5.4	698	54	38	34	13	50	Exceptional

Table 5. Continued.

Stream/ River Mile	No. Qual. Taxa	QCTV ^b	Qualitative Evaluation			Narrative Evaluation ^f
			Qual. EPT ^a	Relative Density	Predominant Organisms	
<i>Fish Creek -1995</i>						
0.3	52	40.9	18	Moderate	Caddisflies and mayflies	Exceptional
<i>Fish Creek -1992</i>						
0.3	26	42.9	12	Moderate	Midges and caddisflies	Good

Ecoregional Biocriteria: Eastern Corn Belt Plains (ECBP)

(from OAC 3745-1-07, Table 7-15)

<u>INDEX</u>	<u>WWH</u>	<u>EWH</u>
ICI	36	46

- ^a - EPT= total Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies) taxa richness.
- ^b - Qualitative Community Tolerance Value (QCTV) derived as the median of the tolerance values calculated for each qualitative taxon present.
- ^c - The qualitative narrative evaluation is based on best professional judgement utilizing sample attributes such as taxa richness, EPT richness, and QCTV score and is used when quantitative data is not available to calculate the Invertebrate Community Index (ICI) scores.
- * - Significant departure from ecoregional biocriterion (>4 ICI units); poor and very poor results are underlined.
- ns - Nonsignificant departure from WWH or EWH biocriterion (≤ 4 ICI units).

Fish Community

A total of 2,582 fish representing 42 species and four hybrids were collected from Fish Creek between August and September, 1997. Relative numbers and species collected per location is presented in Appendix Table 4. Physical habitat scores from 1997 are presented in Appendix Table 5. Sampling locations in Indiana (RM 21.6 - RM 7.5) and in Ohio at RM 5.4 were evaluated using Ohio's Exceptional Warmwater Habitat IBI and MIwb biocriteria. The site at RM 0.3 was evaluated using Ohio Warmwater Habitat IBI and MIwb biocriteria established for the Eastern Corn Belt Plains ecoregion.

Creek chub (18.8%), northern hog sucker (11.3%), and green sunfish (10.5%) predominated the catch numerically, while common carp (33.0%), white sucker (14.0%), and northern hog sucker (13.9%) predominated in weight. Fish highly tolerant of pollution made up 42.5% of the total catch in Fish Creek, the highest percentage in the last three monitoring years (1994, 1995 and 1997).

Pollution intolerant fish species collected in the study area included black redhorse, hornyhead chub, river chub, silver shiner, rosyface shiner, stonecat madtom and brindled madtom. These species together comprised 7.2% of the catch within the Fish Creek study area.

The fish communities at three sites (RMs 21.6-8.3) upstream from the diesel spill ditch exhibited marginally good to good performance (Table 6, Figure 2). All of the IBI and MIwb scores significantly departed from the EWH biocriterion. Average IBI and MIwb scores for the three upstream sites were 39.3 and 8.2, respectively.

Sampling results from two sites (RMs 7.5 and 5.4) in Fish Creek located immediately downstream from the diesel fuel spill area yielded fish communities in the marginally good to good range. Both of the IBI and MIwb scores significantly departed from their respective EWH biocriteria. Average IBI and MIwb scores for the two immediately downstream sites were 40.5 and 8.5, respectively.

Performance of the fish community at RM 0.3 was reduced in comparison to sampling stations upstream. Both the IBI (30) and MIwb (7.0) scores were below WWH ecoregional expectations. Diminished biological performance reflected habitat influences. Though physical habitat at this station was generally good, substrates were predominantly unstable and shifting, consisting mainly of sand. This type of substrate tends to be naturally limiting, providing less than optimal refugia for streambed dwelling organisms. In addition, the lack of well-defined riffles reduces the potential for typical populations of pollution sensitive darters.

The lower IBI scores in Fish Creek in 1997, when compared to previous years, particularly in the upstream reaches, appears to be at least partly associated with a heavy silt layer covering the bottom substrates. The pervasive heavy silt layer encountered during 1997 was the most extensive observed during the last three sampling years (1994, 1995 and 1997). Fine grained sediment constitutes a major environmental factor in the degradation of stream fisheries (Waters 1995).

Table 6. Fish community indices from Fish Creek, 1997 - 1991, based on pulsed D.C. electrofishing at sites sampled by Ohio EPA. Sites were sampled using wading methods. Relative number and weight are per 0.3 km. Fish Creek has a WWH aquatic life use designation in the Ohio Water Quality Standards from RM 2.4 (County Road 3) to the mouth, and an EWH use designation from RM 5.6 (State Line) to RM 2.4. Sampling locations in Indiana were evaluated using Ohio's Exceptional Warmwater Habitat biocriteria.

<i>Stream</i> RM	Sampling Method	Mean # Species	Total # Species	Mean Relative Number	Mean Relative Weight(kg)	QHEI	Mean Modified Index of Well Being	Mean Index of Biotic Integrity	Narrative Evaluation ^a
<i>Fish Creek (1997)</i>									
21.6	Wading -2	20.5	23	527	33.2	75.0	8.5*	36*	Good/Marg. Good
14.3	Wading -2	24.0	31	271	19.4	78.0	8.1*	39*	Marginally Good
8.3	Wading -2	21.0	23	275	35.8	76.0	8.2*	43*	Marg. Good/Good
7.5	Wading -2	19.5	22	230	22.2	73.5	8.3*	39*	Good/Marg. Good
5.4	Wading -2	26.0	31	437	30.4	75.0	8.7*	42*	Good
0.3	Wading -2	19.0	24	182	15.3	69.0	7.0*	30*	Fair
<i>Fish Creek (1995)</i>									
21.6	Wading -2	22.0	25	651	26.8	72.5	8.3*	45*	Good
14.3	Wading -2	28.5	34	360	34.2	77.0	7.9*	48 ^{ns}	M. Good/V. Good
8.3	Wading -2	25.0	29	694	20.2	75.0	8.5*	46 ^{ns}	Good/Very Good
7.5	Wading -2	25.0	28	410	31.4	73.0	8.1*	39*	Marginally Good
5.4	Wading -2	23.5	26	727	6.3	74.0	8.7*	43*	Good
0.3	Wading -2	19.0	23	308	19.5	64.0	7.0*	41	Fair/Good
<i>Fish Creek (1994)</i>									
21.7	Wading -2	22.5	26	819	11.6	76.0	8.1*	40*	Marg. Good
14.3	Wading -2	27.0	34	411	60.8	71.0	7.5*	42*	Fair/Good
8.3	Wading -2	26.0	28	429	38.3	68.0	9.3 ^{ns}	47 ^{ns}	Very Good
6.5	Wading -2	25.0	27	903	31.2	71.5	8.9 ^{ns}	43*	Very Good/Good
5.4	Wading -2	23.5	26	617	9.6	71.0	8.5*	41*	Good
0.3	Wading -2	19.5	24	457	15.1	64.0	7.6*	41	Fair/Good
<i>Fish Creek (1992)</i>									
30.5	Wading -2	16.5	17	4,777	14.9	42.5	NA	44	Good
5.4	Wading -2	26.5	30	587	21.8	77.0	9.3 ^{ns}	44*	Very Good/Good
0.2	Wading -2	21.5	26	695	42.7	65.0	7.5*	43	Fair/Good
<i>Fish Creek (1991)</i>									
5.4	Wading -1	27	-	572	-	79.0	-	52	Exceptional

Ecoregion Biocriteria: Eastern Corn Belt Plains (ECBP)
(from Ohio Administrative Code 3745-1-07, Table 7-15)

<u>INDEX</u>	<u>WWH</u>	<u>EWH</u>	<u>MWH</u> ^b
IBI - Wading	40	50	24
MIwb - Wading	8.3	9.4	6.2

- ^c Significant departure from ecoregional biocriterion (>4 IBI units, >0.5 MIwb units); poor and very poor results are underlined.
- ^{ns} Nonsignificant departure from biocriterion (\leq 4 IBI units, \neq 0.5 MIwb units).
- ^a Narrative evaluation is based on MIwb and IBI scores.
- ^b Modified Warmwater Habitat for channel modified areas.
- NA Headwater site; MIwb is not applicable.

Figure 2. ICI/IBI/MIwb

done

Biomarker Results

Liver and bile samples from common carp were collected at five sampling locations (no samples collected at RM 21.7) during 1997 to detect physiological and biochemical responses to various chemical and environmental exposures. Two indicators reported here are ethoxyresorufin-o-deethylase (EROD) activity and bile metabolites (Table 7). EROD activity is an indicator of the induction of hepatic detoxification systems quantified by measures of a class of metabolic enzymes that are induced by planar xenobiotics such as polycyclic aromatic hydrocarbons and halogenated hydrocarbons. A common carp score below 50 pmol EROD/mg protein is a conservative indication of non-induction. Scores greater than 50 for common carp indicate induction and are a measure of the exposure to these contaminants and detoxification activity by fish.

Bile samples were analyzed for bile metabolites as indicators of exposure to polycyclic aromatic hydrocarbons (PAHs) and to determine relative amounts of exposure among different sites. PAHs with four or more condensed benzene rings are often mutagenic and/ or carcinogenic. The prevalence of neoplasia (*i.e.*, tumors or abnormal tissue growth) in fish has been associated with elevated PAH levels in sediments. Due to their hydrophobic and lipophilic nature, PAHs in aquatic environments rapidly become associated with suspended particles and particles deposited in sediments (McElroy *et al.* 1989). Bioavailability of these PAHs can be addressed by measuring contaminant levels of their metabolites in aquatic organisms. In fish, metabolites of PAHs accumulate in the bile. Two wavelength pairs were used to measure fluorescence in bile, one pair is more sensitive for detecting benzo (a) pyrene (B[a]P)-type compounds and the other for naphthalene (Naph)-type compounds. The B(a)P-type metabolites are generally associated with combustion by-products. The Naph-type metabolites are associated with oil contamination. For common carp, B(a)P -type and Naph-type metabolite scores at or below 0.40 and 130 ug PAH equivalent/mg protein, respectively, are considered reference levels (Cormier *et al.* 2000).

The EROD and bile metabolite results from Fish Creek in 1997 showed no significant difference between sampling locations. All 26 fish tested had EROD levels below the reference level of 50 pmol EROD/mg protein (Table 7). B[a]P-type and Naph-type metabolites for the same fish showed no significant difference between the sites. All bile metabolite values were below reference values (Table 7, Figure 3).

Table 7. Ethoxyresorufin-o-deethylase (EROD) activity and bile metabolite (B[a]P-type and Naph-type) results from common carp collected in the Fish Creek study area, September 15-16, 1997.

River Mile	Total Length (mm)	Weight (grams)	Sex	EROD (pmol /mg protein)	B(a)P-Type (ug PAH Equiv. /mg protein)	Naph-Type (ug PAH Equiv. /mg protein)
14.3	425	944	Male	3.4063	0.1558	39.3
14.3	470	1450	Female	1.1587	0.1721	99.8
14.3	584	2500	Female	1.1470	0.1598	90.6
14.3	432	1100	Male	3.9765	0.1649	92.0
14.3	457	1350	Male	9.1094	0.2027	128.8
14.3	470	1300	Male	6.1920	0.1659	81.5
14.3	540	1950	Male	6.5267	0.2303	105.9
14.3	533	2050	Female	1.3214	0.1758	94.4
14.3	476	2050	Female	1.2988	0.1380	49.3
8.3	286	284	Male	9.0780	0.0617	28.9
8.3	432	1050	Male	8.0666	0.0638	34.2
8.3	521	1850	Female	1.1225	0.0652	32.4
8.3	648	3975	Female	1.1701	0.1309	40.6
7.5	533	2000	Male	11.6678	0.1995	75.7
7.5	368	580	Female	9.4426	0.0862	46.0
5.4	400	778	Male	7.2740	0.1399	107.6
5.4	394	792	Male	3.4884	0.1784	87.4
5.4	387	826	Female	6.6692	0.1024	40.2
5.4	343	540	Female	36.5743	0.1119	51.7
5.4	235	220	Unkno	7.0681	0.0955	48.4
5.4	514	1700	Male	8.2486	0.1192	78.9
5.4	680	4700	Female	3.3953	0.1244	36.3
0.3	571	2400	Female	1.2398	0.0745	43.6
0.3	444	1100	Male	7.1781	0.1373	46.8
0.3	533	1800	Male	5.5609	0.1680	74.6
0.3	457	1200	Female	1.2837	0.0916	55.9

Figure 3. Naph and Bap figure - Done

References

- Cormier, S.M., E.L.C. Lin, F. Fulk, and B. Subramanian. 2000. Estimation of exposure criteria for biliary polycyclic aromatic hydrocarbon metabolite concentrations in white suckers (*Catostomus commersoni*). *Environ. Toxicol. Chem.* 19: 1120-1126.
- Goede, R.W. 1988. Fish health/ condition assessment procedures. Procedures manual and color atlas of autopsy classification categories. Utah Division of Wildlife Resources.
- Lin, E.L.C., S.M. Cormier, and J.A. Torsella. 1996. Fish biliary polycyclic aromatic hydrocarbon metabolites estimated by fixed-wavelength fluorescence: comparison with HPLC-fluorescent detection. *Ecotoxcol. Environ. Safety*, 35, in press.
- Lin, E.L.C., J.E. Klaunig, J.K. Mattox, C.M. Weghorst, B.H. McFarland, and M.A. Pereira. 1989. Comparison of the effects of acute and subacute treatment of phenobarbital in different strains of mice. *Cancer Letters* 48: 43-51.
- MacDonald, D., C. Ingersoll, T. Berger. 2000. Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. *Arch. Environ. Contam. Toxicol.*: Vol.39, 20-31.
- McElroy, A.E., J.W. Farrington, and J.M. Teal. 1989. Bioavailability of polycyclic aromatic hydrocarbons in the aquatic environment, pp. 1-40 (Chapter 1) in U.Varanasi (ed.). *Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment*. CRC Press, Boca Raton, Florida.
- Ohio Environmental Protection Agency. 1987. Biological criteria for the protection of aquatic life: Vol. I. The role of biological data in water quality assessment. Division of Water Quality Planning and Assessment, Ecological Assessment Section, Columbus, Ohio.
- Ohio Environmental Protection Agency. 1989a. Addendum to: Biological criteria for the protection of aquatic life: Vol. II. Users manual for biological field assessment of Ohio surface waters. Division of Water Quality Planning and Assessment, Ecological Assessment Section, Columbus, Ohio.
- Ohio Environmental Protection Agency. 1989b. Biological criteria for the protection of aquatic Life: Vol. III. Standardized field and laboratory methods for assessing fish and macroinvertebrate communities. Division of Water Quality Planning and Assessment, Ecological Assessment Section, Columbus, Ohio.
- Ohio Environmental Protection Agency. 1993a. Biological and water quality study of the St. Joseph River and selected tributaries. OEPA Rept. No. EAS/1993-12-7. Division of Surface Water, Ecological Assessment Section, Columbus, Ohio.
- Ohio Environmental Protection Agency. 1993b. Macroinvertebrate Community Assessment of Fish Creek: 1993 - 1998. Steuben and Dekalb Counties (Indiana) and Williams County (Ohio). Project Summary - Sampling Year 1993. Division of Surface Water, Ecological Assessment Section, Columbus, Ohio.
- Ohio Environmental Protection Agency. 1995. Fish and Macroinvertebrate Study of Fish Creek: 1994. Steuben and Dekalb Counties (Indiana) and Williams County (Ohio). Division of Surface Water, Ecological Assessment Section, Columbus, Ohio.
- Ohio Environmental Protection Agency. 1996. Addendum to Fish and Macroinvertebrate Study of Fish Creek: 1994. Steuben and Dekalb Counties (Indiana) and Williams County (Ohio). Division of Surface Water, Ecological Assessment Section, Columbus, Ohio.
- Pohl, J. and J.R. Fouts. 1980. A rapid method for assaying the metabolism of 7-ethoxy resorufin by microsomal subcellular fractions. *Anal Biochem.* 107: 150-155.
- Rankin, E. T. 1989. The qualitative habitat evaluation index (QHEI): Rationale, methods, and application. Ohio Environmental Protection Agency. Division of Water Quality Planning and Assessment, Ecological Assessment Section, Columbus, Ohio.

Appendix Table 1. ICI metrics and scores for Fish Creek, 1997.

**Appendix Table 2. Raw macroinvertebrate data by river mile for
Fish Creek, 1997.**

Appendix Table 3. IBI metrics and scores for Fish Creek, 1997.

Appendix Table 4. Summary of relative numbers of fish and species collected at each location (by river mile) sampled in Fish Creek, 1997. Relative numbers are per 0.3 km.