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**INDICATORS OF EXPOSURE TO POLYCYCLIC AROMATIC  
HYDROCARBONS (PAH) IN POPULATIONS OF BROWN BULLHEAD  
(*AMEIURUS NEBULOSUS*)**

**Jennifer M. Jones**

**THE OHIO STATE UNIVERSITY**

**Paul C. Baumann**

**U.S. GEOLOGICAL SURVEY**

**Abbreviated Title:**

**Indicators of PAH Exposure in Bullhead**

**Correspondence to:**

**Paul C. Baumann**

**U.S. GEOLOGICAL SURVEY**

**2021 Coffey Road**

**Columbus, Ohio, USA**

## ABSTRACT

Exposure to polycyclic aromatic hydrocarbons (PAH) in fish is indicated by the induction of mixed function oxidase (MFO) activities. Brown bullhead (*Ameiurus nebulosus*) from PAH-contaminated sites in the Black River, Ohio and Hamilton Harbour, Ontario have a history of hepatic carcinoma. Sediments from these sites have been found to be carcinogenic and mutagenic in field and laboratory experiments. Bullhead were collected using fyke nets from these sites as well as a relatively uncontaminated site, Old Woman Creek National Estuarine Reserve near Huron, Ohio. A hepatic MFO assay for 7-ethoxyresorufin O-deethylase (EROD) was used to determine catalytic activity and liver somatic index (LSI) was examined. Bullhead from PAH-contaminated sites showed higher tumor prevalence, elevated EROD activity, and increased LSI when compared with fish from the reference site. In addition, these indicators of PAH exposure in fish from the Black River have shown significant improvements following remediation at that site.

INDEX WORDS: Polycyclic aromatic hydrocarbons, EROD, Liver Somatic Index

## INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) are petroleum or combustion-generated pollutants that are persistent in industrialized areas of aquatic systems in the United States, including the Great Lakes (Melancon *et al* 1987). This contamination has generated much concern due to the potential mutagenic and carcinogenic properties of these compounds (Payne *et al* 1988). PAH in sediments have been linked to neoplastic diseases in wild fish populations (Harshbarger and Clark 1990 and Baumann 1992a).

Since many PAH induce the hepatic mixed function oxidase (MFO) system in fish, MFO activity can be a valuable monitoring tool for assessing PAH exposure in aquatic animals, as reviewed by Payne *et al* (1987). Induction of ethoxyresorufin O-deethylase (EROD) activity is a generally accepted indicator of induction of cytochrome P4501A (CYP1A), the major PAH-inducible form in fish (Stegeman 1992). The EROD assay based on the original method of Burke *et al* (1977) has been modified into a highly sensitive, simple, and economical method of indicating exposure to inducing contaminants (Klotz *et al* 1984). This method centers around the enzymatic O-deethylation of the substrate 7-ethoxyresorufin into resorufin, which can be measured using a fluorescence spectrophotometer (Hodson *et al* 1991).

Adams *et al* (1990) found a correlation between induction of MFO enzymes and general health indices, including the liver somatic index (LSI), in redbreast sunfish. Levine *et al* (1995) found LSI to be a sensitive indicator of the physiological condition in gizzard shad. They recommend the use of this index in conjunction with enzymatic MFO analysis to assess the exposure response in fish inhabiting polluted environments.

In this study, we compared the induction of MFO, measured as EROD activity, liver somatic index, and tumor incidence in brown bullhead (*Ameiurus nebulosus*) from three locations over two years. The Black River, a tributary of Lake Erie near Lorain, Ohio, has had tumor epizootics in brown bullhead linked to sediment PAH contamination documented since the early 1980's (Baumann and Harshbarger 1985). Remedial dredging of these sediments was undertaken in 1990. Brown bullhead from Hamilton Harbour in western Lake Ontario also have high tumor rates, and sediments there are highly contaminated with PAH (Metcalf *et al* 1988). This site has not undergone remedial dredging. These populations were compared with a population from a less contaminated reference site, Old Woman Creek National Estuarine Preserve, near Huron, Ohio.

## METHODS

### Sampling sites

The Black River flows into Lake Erie at Lorain, Ohio. Approximately 5.5 km upstream is the location of the USS/KOBE Steel plant, which has five outfalls within a 2 km stretch of the river (Baumann 1995). In the early 1980's sediments near the coking facility outfall were highly contaminated with PAH (Baumann et al 1982). Black River sediment extracts were found to be carcinogenic when tested by mouse skin painting (Black *et al* 1985). After the closing of the coking facility in 1983, the level of PAH in surficial sediments in the Black River declined (Baumann and Harshbarger 1995). In 1990, a dredging operation was undertaken to remove these contaminated sediments from the vicinity of the outfall.

Hamilton Harbour is an isolated embayment in western Lake Ontario. This area is industrialized, with two steel mills discharging treated wastewaters directly into the harbour. Sediments in the harbour are contaminated with PAH and have been found to induce hepatocellular carcinomas in rainbow trout (*Oncorhynchus mykiss*) in the laboratory (Metcalf *et al* 1988). Balch *et al* (1994) detected mutagenic activity in sediment extract from Hamilton Harbour with the Ames test as well as carcinogenicity using an in vivo bioassay with rainbow trout.

Old Woman Creek is located about 32 km west of the Black River near Huron, Ohio. The lower reaches of the creek flow into Lake Erie and are part of the Old Woman Creek National Estuarine Preserve. It has a small, primarily agricultural watershed, with low-level PAH contamination at railway and highway bridges (Johnston and Baumann 1989). This system served as a non-industrialized reference site for this study.

### Sampling of fish

Brown bullhead were collected by overnight sets of fyke nets with 12 mm or 25 mm mesh and 9-16 m leads. Sampling occurred in the Black River and Old Woman

Creek in May and September of 1994 and in May and October of 1995. Fish were collected from Hamilton Harbour in September of 1994 and October of 1995 using overnight fyke and gill nets. Brown bullhead of 250 millimeters or larger, corresponding to age three or older, were collected. Fish were placed in a holding tank for transport to the laboratory. Individual fish were then transferred to a smaller container and tricaine methanesulfonate (MS 222) was added to the water (100 mg/L) to anesthetize fish. A foam bed was used to immobilize the fish and blood was collected for use in other studies. Fish were then euthanized by cervical dislocation. Each fish was weighed to the nearest gram and total length was measured to the nearest millimeter.

Fish were then examined for external abnormalities following the method of Baumann (1992b). Pectoral spines were removed and were used to age the fish by the methods of Marzolf (1955) and Scholl (1968) as described by Baumann *et al* (1990). Gonads were removed and weighed. Livers were removed after disposal of the intact gallbladder and weighed. Grossly visible liver nodules were recorded to determine the incidence of liver tumors in the three fish populations. Portions of 1-2 grams were immediately frozen in liquid nitrogen using 1-ml cryovials and stored at -80 C until analysis. The remaining liver was preserved in 10% formalin for histopathological analysis by Dr. John Harshbarger at the Smithsonian Institution.

#### EROD Analysis

Hepatic MFO analysis was carried out in the laboratory of Dr. Kelly Munkittrick at the Department of Fisheries and Oceans, Burlington, Ontario. Livers were thawed on ice and homogenized in 20 uM HEPES (pH 7.5) and 150 uM KCl at a volume of 4 ml buffer to 1 g liver. The homogenate was then centrifuged to obtain the 10,000-g post-mitochondrial fraction (S9 fraction). The activity of 7-ethoxyresorufin-O-deethylase (EROD) in the postmitochondrial fraction was determined fluorometrically by measuring the production of resorufin from 7-ethoxyresorufin (Pierce Chromatographics), using a Perkin-Elmer LS-50 spectrofluorometer with excitation wavelength of 530 nm and emission wavelength of 582 nm. The final reaction mixture contained 0.1 M HEPES at

pH 7.8, 12.8 uM MgSo<sub>4</sub>, 0.03 uM BSA, 0.8 uM NADPH (Sigma), and 100 ul of post-mitochondrial liver homogenate in a volume of 1.34 ml. The reaction was initiated by the addition of 20 uL of 7-ethoxyresorufin (0.022 mg/ml) and was stopped after 10 minutes by the addition of 3 ml of methanol. Each sample was run in triplicate at 25 C along with a blank. EROD activity was expressed as picomoles of resorufin per minute per milligram of protein. A modification of the method of Lowry *et al* (1951) was used to measure the post-mitochondrial protein with absorbance wavelength of 600 nm on a UV-Vis Shimadzu spectrophotometer.

#### Liver Somatic Index

The liver somatic index (LSI) was calculated for each fish as [liver weight (g) / body weight (g)] \* 100 (Adams and MacLean 1985).

#### Statistical analysis

All EROD, LSI, and condition factor values were expressed as means  $\pm$  standard error of the mean (SEM) and were log-transformed prior to statistical treatment to reduce heteroscedasticity. The nonparametric Kruskal-Wallis one-way analysis of variance (ANOVA) was performed to test for differences in values of all parameters among sites, sexes, and times of year. The chi-square test was used to analyze differences in tumor prevalence among sites. SYSTAT was used for all analyses. The level of statistical significance was set at  $p < 0.05$ .

## RESULTS

Since the closing of the coking facility at the USX Steel plant in 1983, the PAH contamination of surficial sediment in the Black River has declined (Table 1). Sediment data from 1992, two years after large-scale dredging around the outfalls, show a higher level of PAH contamination than in 1987. 1994 sediment data show lower PAH levels than 1992, although still somewhat elevated from the 1987 levels.

Table 1. Black River sediment concentrations (ug/g dry sediment) of various PAH compounds.

Compound	1980 <sup>a</sup>	1982 <sup>b</sup>	1984 <sup>c</sup>	1987 <sup>d</sup>	1992 <sup>e</sup>	1994 <sup>f</sup>
Acenaphthylene	40.0	8.00	17.0	0.08	1.10	0.013
Acenaphthene	36.0	7.20	2.50	0.14	N/A	0.055
Phenanthrene	390.0	100.0	52.0	0.73	2.10	1.86
Fluoranthene	220.0	76.0	33.0	0.79	3.35	3.46
Pyrene	140.0	56.0	24.0	0.93	2.95	2.86
Benz(a)anthracene	51.0	23.0	11.0	0.37	2.40	0.313
Chrysene/triphenylene	51.0	25.0	10.0	0.37	2.20	N/A
Benzo(a)pyrene	43.0	21.0	8.80	0.24	2.65	0.237
Benzofluoranthenes	75.0	37.0	15.0	0.58	N/A	0.828
Benzo(ghi)perylene	24.0	13.0	5.40	0.03	N/A	N/A
Indeno(1,2,3-cd)pyrene	26.0	15.0	6.40	0.01	1.75	0.195
TOTAL <sup>g</sup>	1096.0	381.2	185.1	4.27	18.5	9.826

<sup>a</sup>Baumann et al (1982)

<sup>b</sup>West et al (1985)

<sup>c</sup>Fabacher et al (1988)

<sup>d</sup>Smith et al (1994)

<sup>e</sup>Black River Remedial Action Plan (1992)

<sup>f</sup>Munkittrick et al (unpublished data)

<sup>g</sup>Total PAH content represents the sum of all values reported in the table.

Table 2. Hamilton Harbour sediment concentrations (ug/g dry sediment) of various PAH compounds<sup>a</sup>.

Compound	concentration
Anthracene	14.5
Benz[a]anthracene	40.6
Benzo[a]pyrene	44.9
Benzo[e]pyrene	27.3
Benzo[ghi]perylene	14.3
Chrysene	40.5
Fluoranthene	80.3
Fluorene	7.1
Ideno[1,2,3-cd]pyrene	22.2
Naphthalene	93.9
Phenanthrene	55.6
Pyrene	55.2
TOTAL <sup>b</sup>	496.4

<sup>a</sup>Marvin *et al* (1993)

<sup>b</sup>Total PAH content represents the sum of all values reported in the table.

Sediments in Hamilton Harbour have higher levels of PAH than Black River sediments (Table 2). An extensive survey of the area by Murphy *et al* (1990) indicates that PAH levels in the Harbor are greater than 800 ug/g in many areas and up to 1400 ug/g in hotspots near outfall pipes of the Stelco steel facility.

Sediments collected from localized areas of Old Woman Creek have been found to have low-level PAH contamination (Johnston and Baumann 1989) (Table 3). In particular, a railroad tressel in one area of the creek may be receiving PAH from pilings and discarded railroad ties coated with creosote, while another area, near a highway bridge, could be receiving petroleum runoff. Even in these areas, the PAH concentration in sediment from Old Woman Creek in 1985 was two orders of magnitude less than that in the Black River in 1984 (Johnston and Baumann 1989).

#### Tumor Incidence

Since the closing of the steel plant coke facility at the Black River in 1983, the incidence of hepatic neoplasm has declined in brown bullhead there. Histopathological evaluation of livers from fish collected from the Black River in 1982 showed that 60% of fish exhibited liver neoplasms, whereas in 1987 incidence had declined to 32.5% (Baumann and Harshbarger 1995). In 1992, liver neoplasia incidence had increased to 58%, presumably as a result of the dredging in 1990. In 1993, 61% of fish had liver neoplasms (Baumann and Harshbarger, personal communication).

Histopathological evaluation of livers from fish collected for this study was only available for the spring 1994 sample. Fish from the Black River exhibited liver neoplasia incidence of 14 %, significantly less than in 1993 using chi-square at  $p < 0.05$  (Baumann and Harshbarger, personal communication).

The incidence of grossly visible liver tumors in fish collected from Old Woman Creek was consistently less than 1%, while fish from both the Black River and Hamilton Harbour had significantly higher liver tumor rates (Figure 1). There seems to be an effect of season on liver tumor incidence at the Black River. Fish collected in the fall from the Black River exhibited more liver tumors than did those in the spring in both

Table 3. Old Woman Creek sediment concentrations (ug/g dry sediment) of various PAH compounds<sup>a</sup>.

Compound	concentration
Anthracene	0.013
Benz[a]anthracene	0.078
Benzo[a]pyrene	0.271
Benzo[ghi]perylene	0.076
Chrysene	0.089
Fluoranthene	0.222
Ideno[1,2,3-cd]pyrene	0.051
Phenanthrene	0.047
Pyrene	0.136
TOTAL <sup>b</sup>	0.983

<sup>a</sup>Johnston and Baumann (1989)

<sup>b</sup>Total PAH content represents the sum of all values reported in the table.

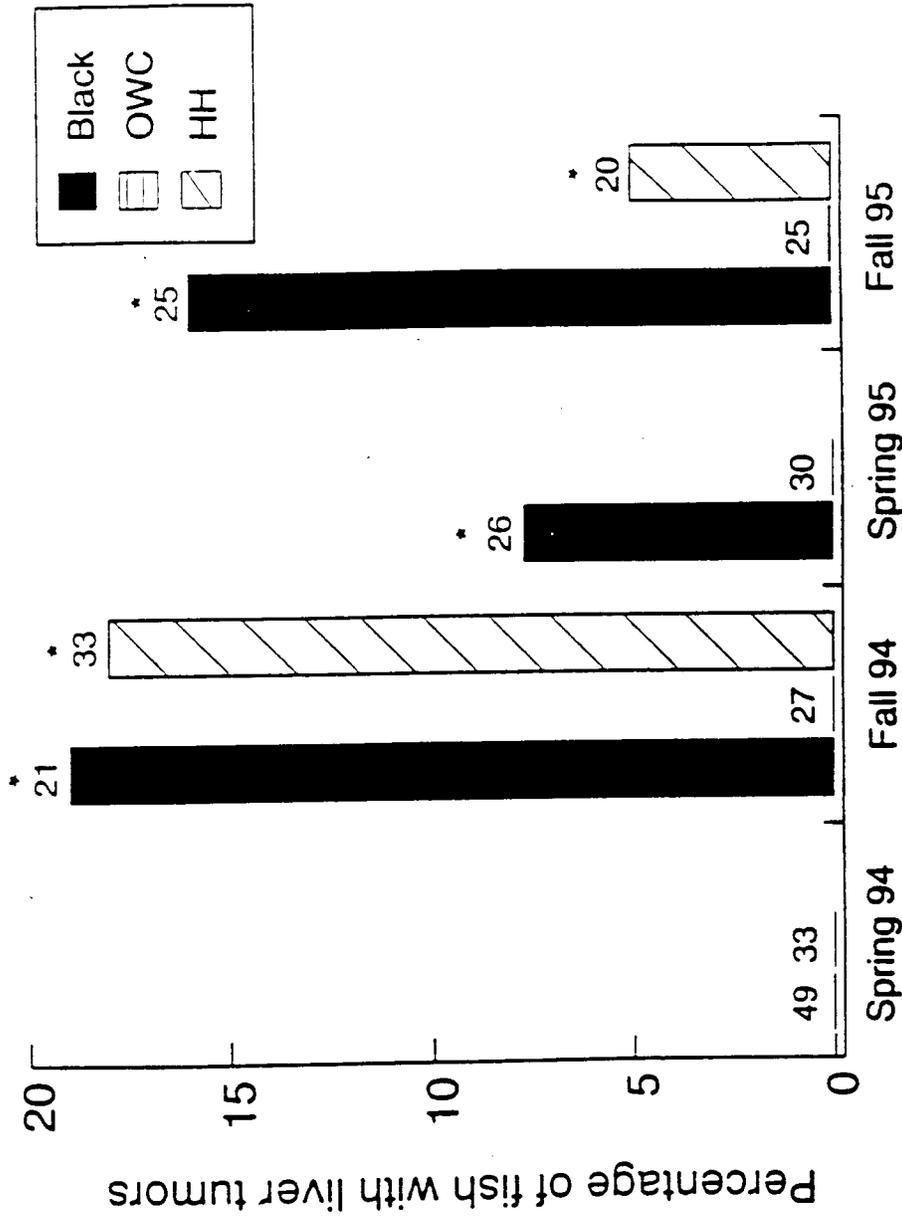


Figure 1. The prevalence of grossly visible liver tumors in brown bullhead collected from the Black River, Old Woman Creek, and Hamilton Harbour. Sites within the same sampling time always appear in the order above. No collections were made in the spring from Hamilton Harbour. \* Indicates significantly greater than Old Woman Creek using chi-square at  $p < 0.05$ . Sample sizes are shown above each bar.

1994 and 1995. There was no apparent effect of gender on tumor incidence at any site.

## EROD

Sampling in both the spring (May) and fall (September and October) at the Black River and Old Woman Creek reference site allowed for comparison of MFO activity during spawning and postspawning periods. In the spring, EROD levels were significantly lower than in the fall for both the Black River and Old Woman Creek ( $p \leq 0.0001$ , Figure 2). Fish from the Black River had significantly greater EROD levels than those from Old Woman Creek in the fall of both years ( $p \leq 0.0001$ ) and in the spring of 1995 ( $p = 0.010$ ). There was no difference in EROD activity in fish from the Black River compared to those from Old Woman Creek in the spring of 1994.

EROD declined significantly in brown bullhead from the Black River from the fall of 1994 to the fall of 1995 ( $p \leq 0.0001$ ). In the fall of 1994, EROD levels in Black River and Hamilton Harbour fish were not significantly different, whereas in the fall of 1995, fish from Hamilton Harbour showed significantly higher EROD activity than those from the Black River ( $p = 0.002$ , Figure 3). Hamilton Harbour fish had significantly higher EROD levels than fish from Old Woman Creek in both 1994 ( $p = 0.001$ ) and 1995 ( $p \leq 0.0001$ ).

There were no differences in EROD activity based on gender at any site, with the exception of the fall 1994 sampling at Old Woman Creek, where females had significantly greater EROD activity than males ( $p = 0.020$ ). Seasonal changes in EROD activity at the Black River throughout the study period were parallel in males and females, with males having slightly higher, but not statistically different EROD levels.

## Population Characteristics and LSI

Brown bullhead collected from the Black River were significantly greater ( $p \leq 0.0001$ ) in length and weight than those from the Old Woman Creek reference site,

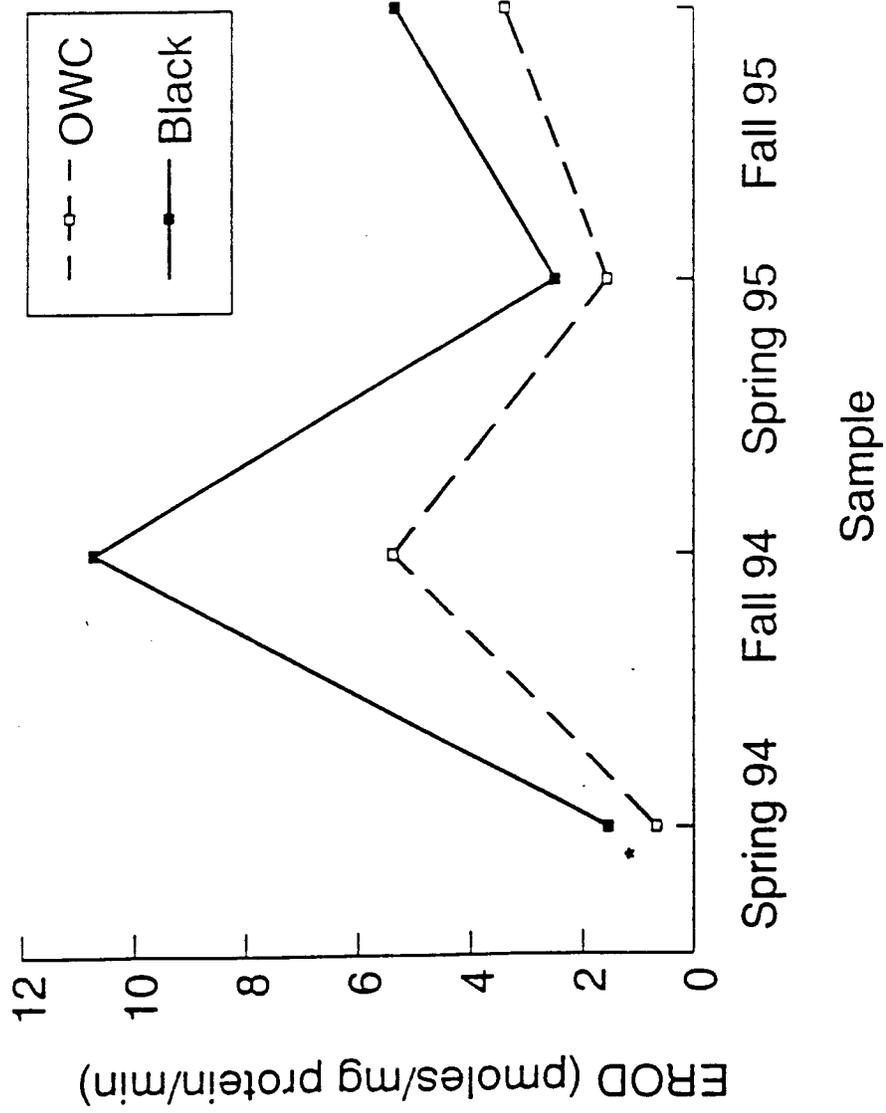


Figure 7. EROD activity in brown bullhead collected from the Black River and Old Woman Creek from the spring of 1994 to the fall of 1995.  
 \* EROD values in the spring of 1994 are not significantly different using ANOVA at  $p < .05$ .

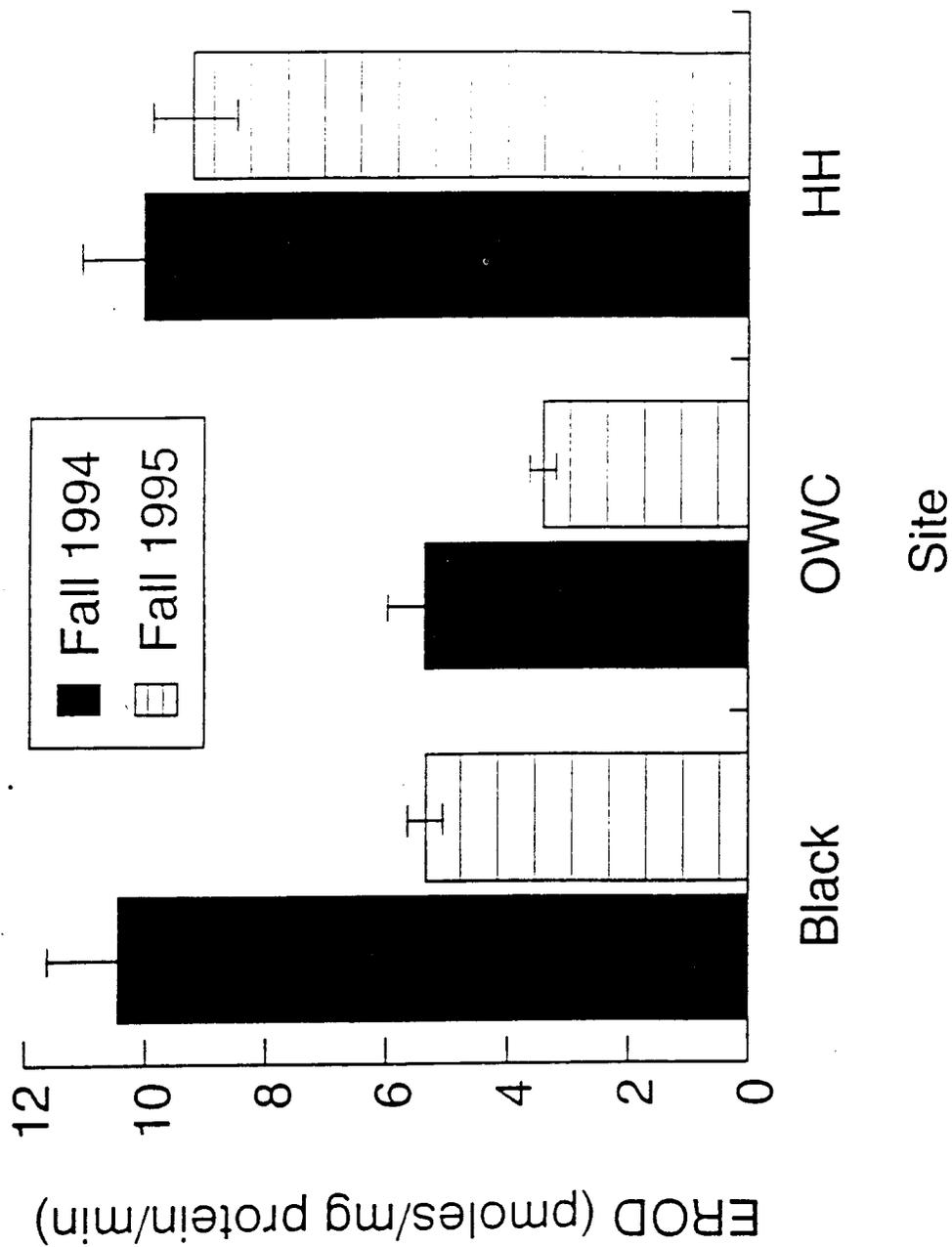


Figure 3. EROD activity in brown bullhead collected from the Black River, Hamilton Harbour, and Old Woman Creek in the fall of 1994 and 1995.

Table 4. Brown bullhead population characteristics for the fall of 1994 and 1995 at Old Woman Creek, the Black River, and Hamilton Harbour. Values represent the means  $\pm$  S.E. Values sharing letters not significantly different using ANOVA at  $p < 0.05$  on log-transformed data.

Year	N	Site	Age	Length (cm)	Weight (g)	Condition Factor	LSI
1994	27	Old Woman Creek	4.0 $\pm$ 0.2 a	26.6 $\pm$ 0.5 a	249.5 $\pm$ 14.6 a	1.3 $\pm$ 0.03 a	1.7 $\pm$ 0.07 a
	20	Black River	3.0 $\pm$ 0.1 b	31.3 $\pm$ 0.7 b	429.6 $\pm$ 22.9 a	1.4 $\pm$ 0.07 a	2.2 $\pm$ 0.1 b
	20	Hamilton Harbour	5.7 $\pm$ 0.6 c	31.3 $\pm$ 0.7 b	392.8 $\pm$ 25.4 a	1.3 $\pm$ 0.04 a	2.1 $\pm$ 0.09 b
1995	25	Old Woman Creek	4.1 $\pm$ 0.2 a	28.6 $\pm$ 0.6 a	309.2 $\pm$ 23.9 a	1.3 $\pm$ 0.02 a	1.8 $\pm$ 0.06 a
	24	Black River	3.7 $\pm$ 0.1 a	34.8 $\pm$ 0.5 b	579.8 $\pm$ 20.4 b	1.4 $\pm$ 0.02 a	2.0 $\pm$ 0.06 a
	20	Hamilton Harbour	4.2 $\pm$ 0.3 a	33.5 $\pm$ 1.6 b	468.9 $\pm$ 19.3 b	1.4 $\pm$ 0.07 a	2.9 $\pm$ 0.1 b

but not in condition factor (Table 4). Black River fish were also younger than fish from Old Woman Creek or Hamilton Harbour. In the fall of both 1994 and 1995, all fish collected from the Black River were of ages 3 or 4. In contrast, Old Woman Creek and Hamilton Harbour had fish as old as age 7 and 10, respectively. There were no significant differences in length, weight, or condition factor between fish from the Black River and Hamilton Harbour.

Fish from both the Black River and Old Woman Creek had higher LSI values in the spring than in the fall, although these values were not statistically significant (Figure 4). LSI was significantly greater in fish from the Black River than in Old Woman Creek in the spring ( $p = 0.001$ ) and fall ( $p = 0.017$ ) of 1994, and in the spring of 1995 ( $p \leq 0.0001$ ). However, there was no significant difference between the two sites in the fall of 1995.

In the fall of 1994, no significant difference was found between LSI from the Black River and Hamilton Harbour; both were significantly higher than Old Woman Creek (Black River at  $p = 0.017$ , Hamilton Harbour at  $p = 0.003$ ) (Table 4). However, in the fall of 1995, LSI was significantly less in fish from the Black River as compared to that from fish in Hamilton Harbour ( $p \leq 0.0001$ ), while there was no difference in LSI between the Black River fish and Old Woman Creek fish. LSI was only weakly correlated with EROD activity (Pearson's correlation coefficient  $r = -.40$ ). There was no correlation between LSI and age. When analyzed by sex, a trend was found toward significantly greater LSI in female fish at all sites in the fall. No clear trend in LSI due to gender was apparent in fish collected in the spring.

## DISCUSSION AND CONCLUSIONS

### Liver tumors

Grossly visible liver tumors in populations of brown bullhead have been described since the early 1980s in the Black River (Baumann *et al* 1990) as well as Hamilton Harbour (Metcalfe *et al* 1988). The types of hepatic lesions found in bullhead

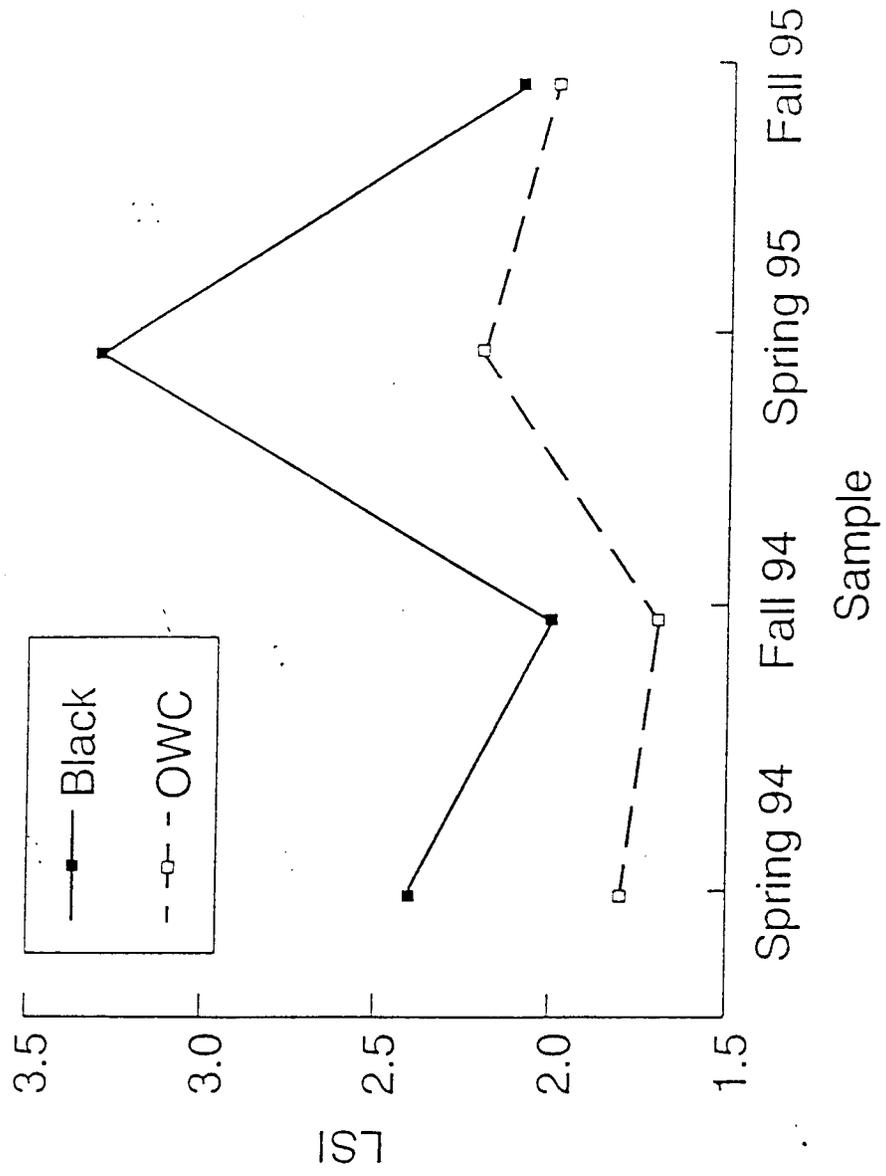


Figure 4. Liver Somatic Index (LSI) in brown bullhead collected from the Black River and Old Woman Creek from the spring of 1994 to the fall of 1995. \*LSI values in the fall of 1995 are not significantly different using ANOVA at  $p < 0.05$ .

from these sites, specifically hepatocellular and cholangiocellular neoplasms, have been strongly linked to PAH exposure (Harshbarger and Clark 1990).

Histopathological evaluation of livers from fish collected from the Black River in the spring of 1994 shows liver neoplasia incidence of only 14 %. While this is statistically greater than the incidence of liver neoplasia in fish from Old Woman Creek, which was less than 1%, it shows a trend toward improving conditions at the Black River, where the neoplasia frequency was 61% in 1993.

Estimates of liver tumor incidence based on gross observations are probably conservative because, unlike cholangiocellular (biliary) lesions, hepatocellular lesions are often not grossly observable (Baumann and Harshbarger 1985). These estimates show liver tumor incidence to have declined in fish from the Black River to 19% in the fall of 1994 and 16% in the fall of 1995.

In contrast, brown bullhead collected from Hamilton Harbour in the mid 1980's exhibited hepatic neoplasms at a much lower rate: 1.6% (Metcalf *et al* 1988) and 6% (Baumann *et al* 1996). This is comparable with tumor incidence at Old Woman Creek, where 5% of fish collected in 1993 exhibited hepatic neoplasms. Estimations based on gross observation indicate a higher incidence of liver tumors in fish from Hamilton Harbour in 1994 (18%) and 1995 (5%), whereas the incidence in fish collected from Old Woman Creek was less than 1% in both spring and fall of 1994 and 1995 (Figure 5). Even so, the incidence of liver tumors in fish from Hamilton Harbour is lower than would be expected given the high levels of PAH contamination there and the apparent older age of the fish collected.

Because the presence of tumors is a chronic response with a latent period between exposure to pollutants and tumor formation, the effects of season on tumor incidence seen at the Black River may be due to sampling bias. In the spring, bullhead from Lake Erie have migrated up the Black River to spawn, and the decreased tumor incidence reported then is probably a result of the dilution of Black River fish with relatively unexposed populations from the lake (Baumann 1992b).

Age of the fish population is another important variable influencing the

probability of finding neoplastic lesions (Baumann *et al* 1990). In the fall of both 1994 and 1995, all fish collected from the Black River were of ages 3 or 4, which may reflect age-related mortality in Black River fish. In contrast, the age of fish from Hamilton Harbour was more evenly distributed, suggesting that fish are living longer there despite the high levels of sediment PAH.

## EROD

Fish from both the Black River and Hamilton Harbour have elevated levels of EROD activity compared to the less contaminated Old Woman Creek, presumably in response to high levels of PAH contamination there. In conjunction with declining levels of PAH in sediments in the Black River following dredging, EROD levels declined from 1994 to 1995. In Hamilton Harbour, where no such remediation has occurred, EROD activity did not decline from 1994 to 1995.

Because MFO enzymes also metabolize endogenous compounds such as steroid hormones, the induction of the MFO system may affect physiological functions such as the reproductive and immune systems, thereby affecting the population as a whole (Munkittrick 1992). For instance, Spies *et al* (1988) found reduced reproductive success in starry flounder exposed to contaminants in San Francisco Bay. Other sublethal effects of PAH exposure, including enlarged livers and liver neoplasia, can shorten the life span of individuals and affect the general stability of a population (Fabacher and Baumann 1985 and Baumann *et al* 1990).

It is important to recognize the limitations of using catalytic enzyme assays to assess the health of a wild fish population. Wild fish populations are exposed to complex environmental contaminant mixtures as well as varying seasonal conditions, resulting in changes in temperature and nutritional resources. All of these factors contribute to the variability of enzyme levels in individual fish. In their study of three species of flatfish in Puget Sound, Collier *et al* (1995) found a correlation between induction of CYP1A measured by ELISA and MFO induction measured by catalytic EROD and AHH assays. EROD and AHH showed a greater range of response than

the immunoassay, but EROD showed much variation due to species and temporal differences. In the present study, emphasis is placed on the comparison of EROD activity in brown bullhead collected from different sites at the same time of year.

Forlin and Celander (1993) found that EROD activity was more sensitive than the induction of CYP1A as measured by ELISA in indicating pollutant exposure. However, Goksoyr (1991) found that cytochrome P4501A protein can become denatured during the handling and storage of samples, resulting in a loss of catalytic activity. At the same time, the antigenic epitopes of the protein were still recognized by the antibodies. Thus, future research efforts should concentrate on immunoassays in conjunction with catalytic assays for CYP1A induction in these fish.

Immunohistochemical techniques using antibodies to CYP1A can also be employed to determine its induction in different cell types and, in effect, trace the route of exposure in the organism. Using these techniques, Collier *et al* (1995) have seen patterns of CYP1A expression that follow cellular patterns of pathological injury, suggesting a mechanistic link between CYP1A induction and neoplasia.

#### Liver Somatic Index

Although EROD was not statistically correlated with LSI, LSI showed a similar response to remediation of PAH contamination by declining significantly in fish from the Black River from 1994 to 1995. It is unknown whether higher LSI values in bullhead from the Black River and Hamilton Harbour are due to liver hypertrophy or hyperplasia. The apparent decline in LSI in fish from the Black River from 1994 to 1995 may indicate that these fish are responding to improving conditions there. Because liver hypertrophy can be acute and essentially reversible (Payne *et al* 1988), it would be of benefit to continue to assess LSI in fish from the Black River to see if the apparent decline in LSI continues over time.

Female fish were found to have greater LSI than males in the fall at all sites. This may be due to the effects of gonadal recrudescence, when fish begin to prepare for spawning in the spring. In the fall, the initiation of vitellogenesis in female fish may

result in increased liver size. Perhaps due to the confounding effects of reproduction, including variability in individual fecundity and time of spawning, no consistent trend was found in LSI with respect to sex.

In a study by Fabacher and Baumann (1985), brown bullhead collected from the Black River in 1981 and 1982 had elevated LSI values (1.7 and 2.7 times, respectively) when compared to a reference site. Jodon (1987) also found LSI in fish from the Black River to be elevated (2 times) compared to LSI in fish from Old Woman Creek in 1985, two years after the coking facility at the Black River was closed. Fish collected from the Black River in 1994 for the present study had LSI elevated only 1.2 times that at Old Woman Creek. Further, fish collected in the fall of 1995 were not significantly different in LSI from those collected at Old Woman Creek. This suggests that in fish from the Black River, LSI is decreasing in parallel with both levels of PAH in surficial sediments and with the incidence of grossly visible tumors.

The importance of LSI values to the population as a whole are dependent on the long term effects of liver enlargement. According to Payne *et al* (1988), prolonged liver enlargement is assumed to be biologically harmful, as evidenced in human toxicological studies where enlarged livers in chronic alcoholics have been associated with extensive cirrhosis and sometimes carcinogenesis. There are indications that cellular proliferation of the liver may increase the activation of promutagens and procarcinogens (Fabacher and Baumann 1985), but the mechanisms by which this occurs are unclear.

Further, much more needs to be learned about the migratory and feeding patterns of brown bullhead. Because habitat conditions are fairly homologous along stretches of the Black River, movement of brown bullhead populations there may be somewhat limited. In addition, contaminated areas of the river around the steel plant outfalls are relatively shallow, especially in the summer when fish are consuming more benthic invertebrates. As a result, Black River fish may be more closely associated with PAH contamination over longer periods of time than in Hamilton Harbour, where areas of high contamination may be less available to fish due to

acutely toxic conditions (Murphy *et al* 1990).

Brown bullhead in Hamilton Harbour probably spend their summers in Cootes Paradise, a shallow, marshy area just to the west of Hamilton Harbour which provides plenty of food in the warm months. In the fall, these fish probably move to the harbour where they have less competition with pike and salmon which remain at the mouth of Cootes Paradise in the winter (V. Cairns, personal communication). While in the harbour, bullhead may stay around warm sewage treatment and effluent outfalls, becoming exposed to PAH contamination there. Even so, fish may be present in these areas for only a short period of time, and may not be inhabiting areas having the most severe contamination. Thus, PAH contamination in Hamilton Harbour may not be as available to fish as that in the Black River.

## CONCLUSIONS

MFO activity, as measured by EROD, appears to be a good indicator of exposure to PAH in these fish. EROD activity in fish from the Black River and Hamilton Harbour in the fall of 1994 was twice that in fish from Old Woman Creek, a relatively uncontaminated site. Coinciding with continually improving conditions at the Black River following remediation, EROD activity in fish from the Black River was not significantly higher than in fish from Old Woman Creek in the fall of 1995. In contrast, EROD continues to be elevated in fish from unremediated Hamilton Harbour.

The usefulness of measuring EROD induction centers around how well it reliably indicates exposure to contaminants. Even though this field study emphasized the comparison of MFO and other responses in fish populations collected at the same time of year, other factors can contribute to variability in these measurements. Other variables, such as temperature and nutrition, are not easily controlled for, but can affect a two-fold change in EROD (K. Munkittrick, personal communication). Another variable that directly affects exposure is fish migration. In addition, wild fish are exposed to complex mixtures of contaminants that differ between sites, not only in composition and

quantity, but also in availability, and these important factors differ temporally within sites.

As conditions continue to improve at the Black River and the level of EROD induction decreases, it will be increasingly important to control variability in EROD for its continued usefulness as an indicator of PAH exposure. In this study, EROD, along with the incidence of liver neoplasia and liver somatic index, are good indicators of exposure to PAH in brown bullhead when used to show differences among sites as well as reflecting improvement in response to remediation.

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Figure 4.

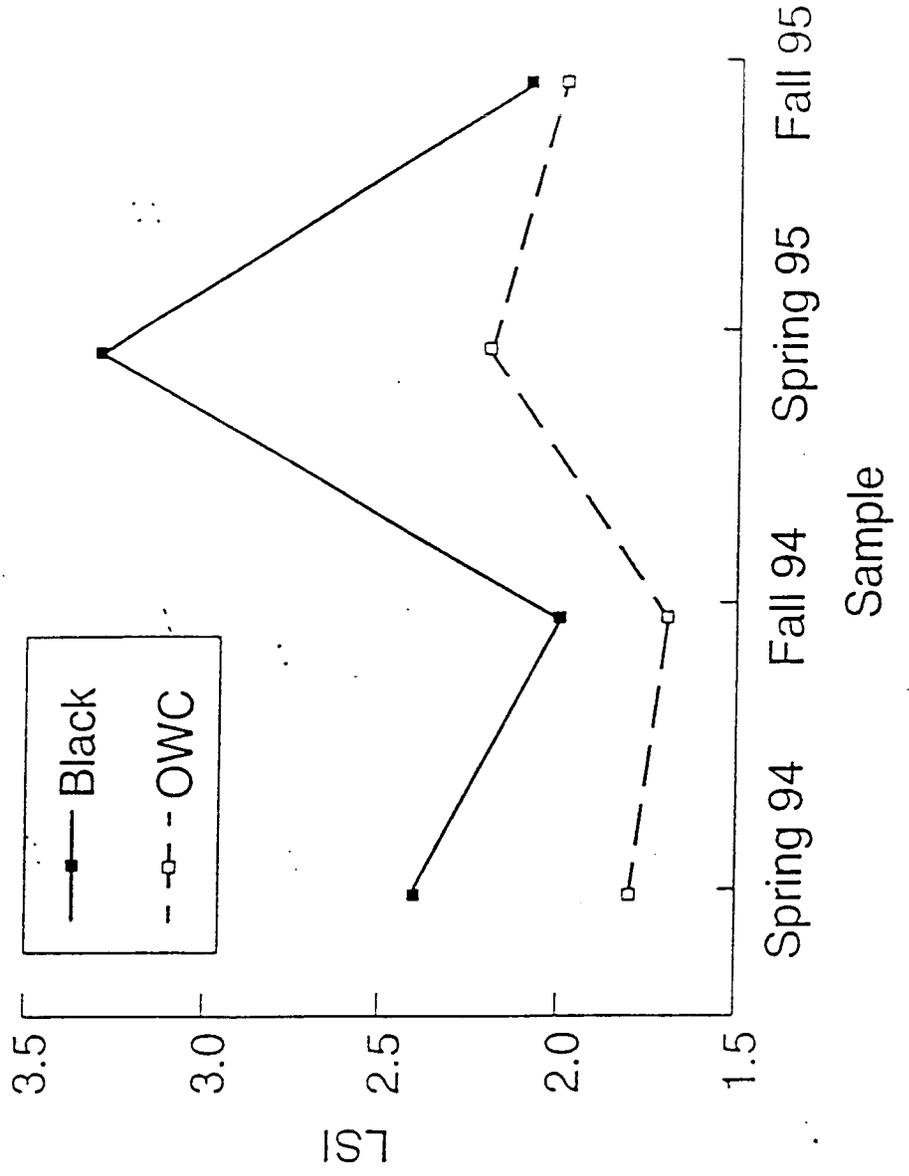


Figure 3.

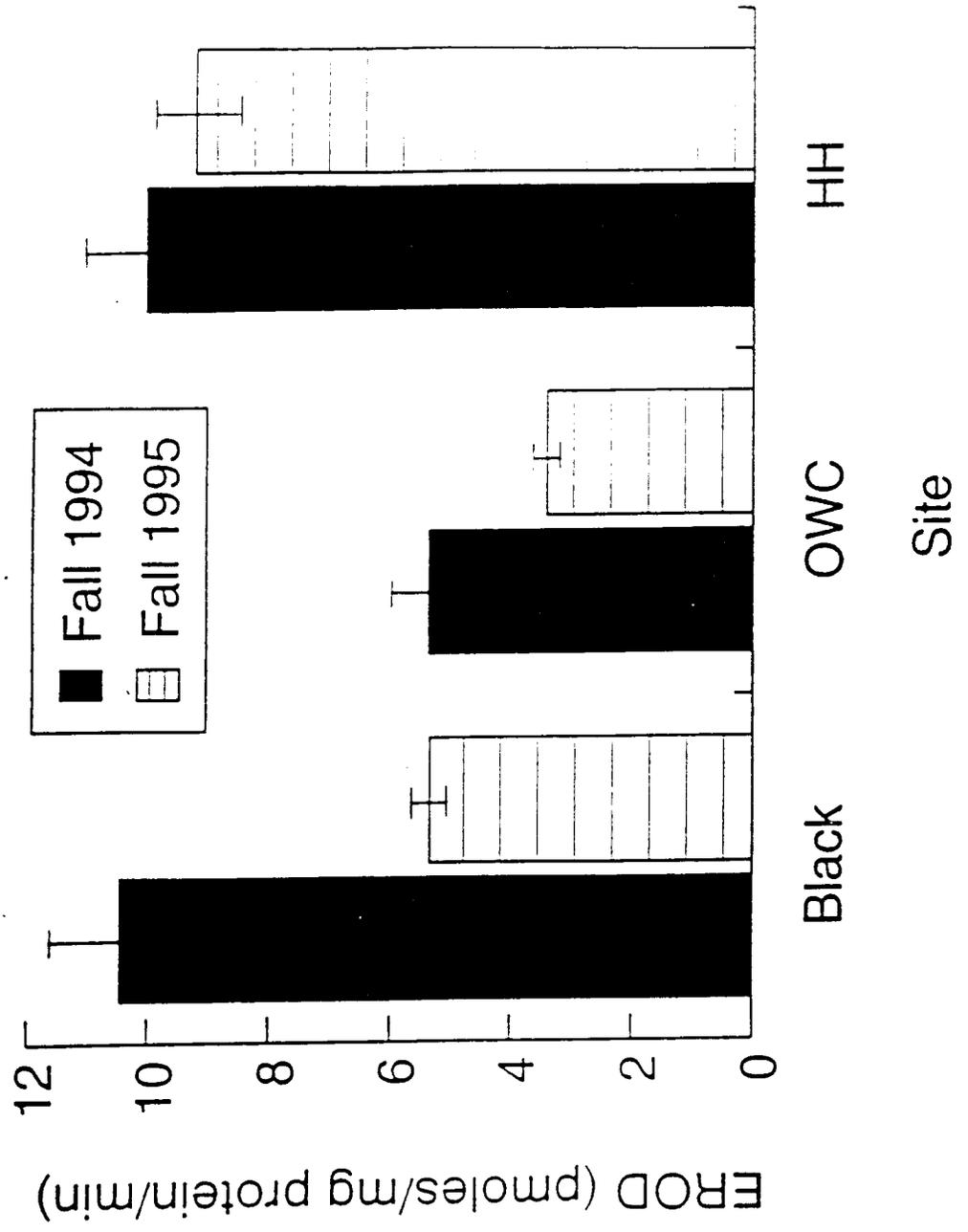


Figure 2.

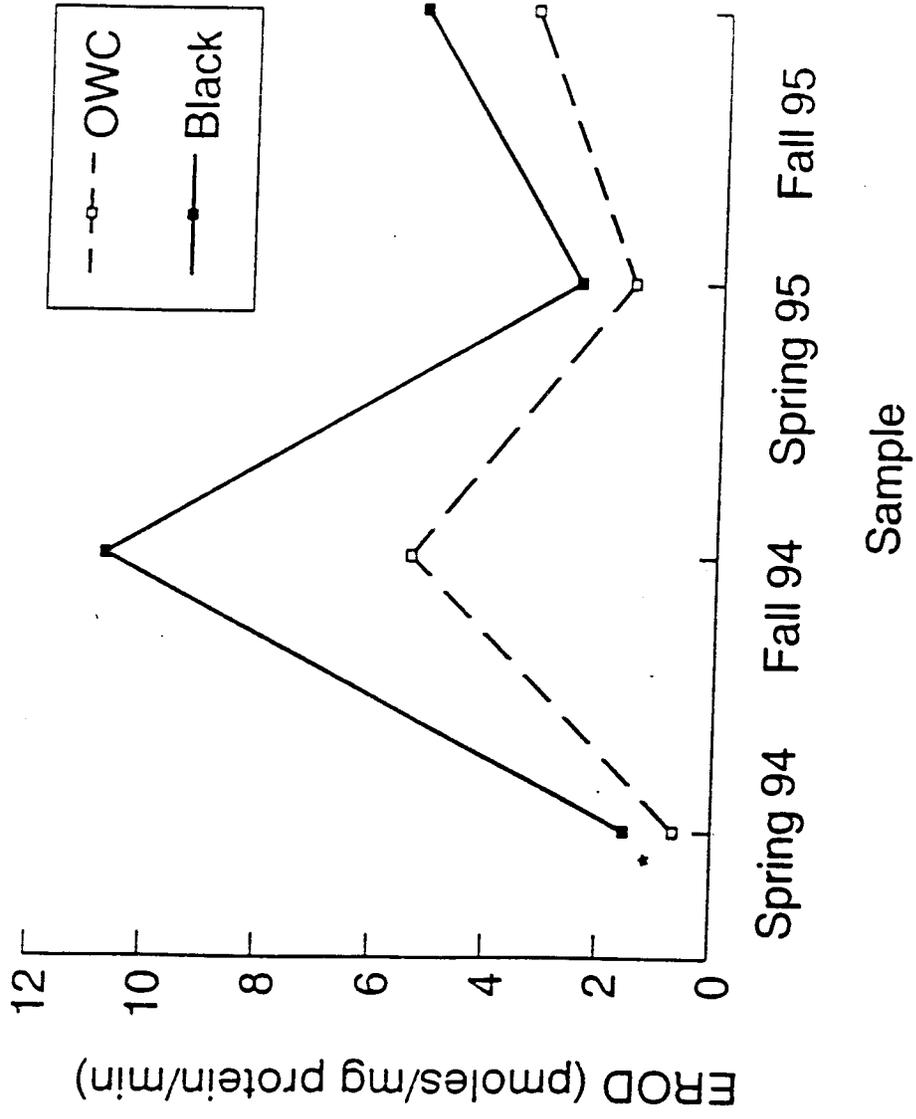


Figure 1.

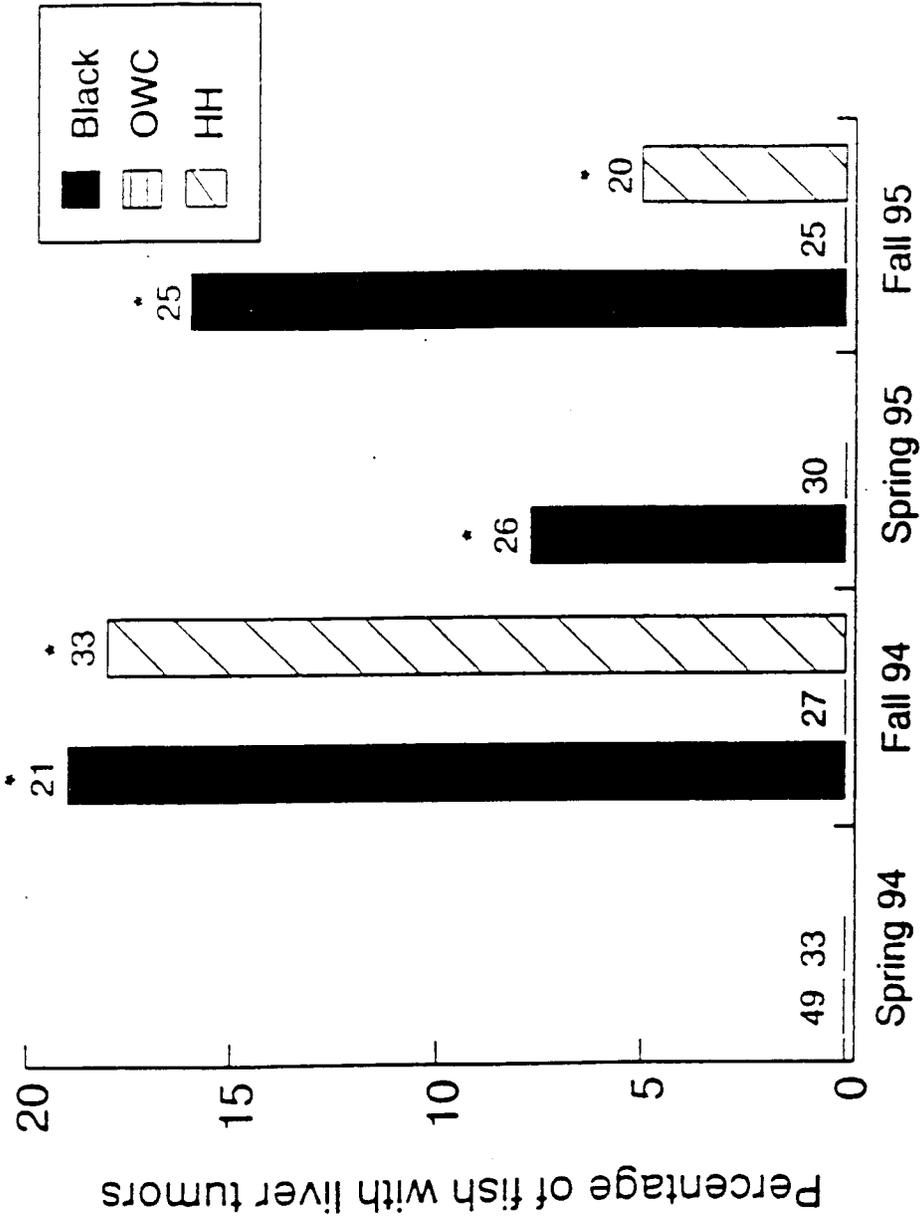


Figure 4.

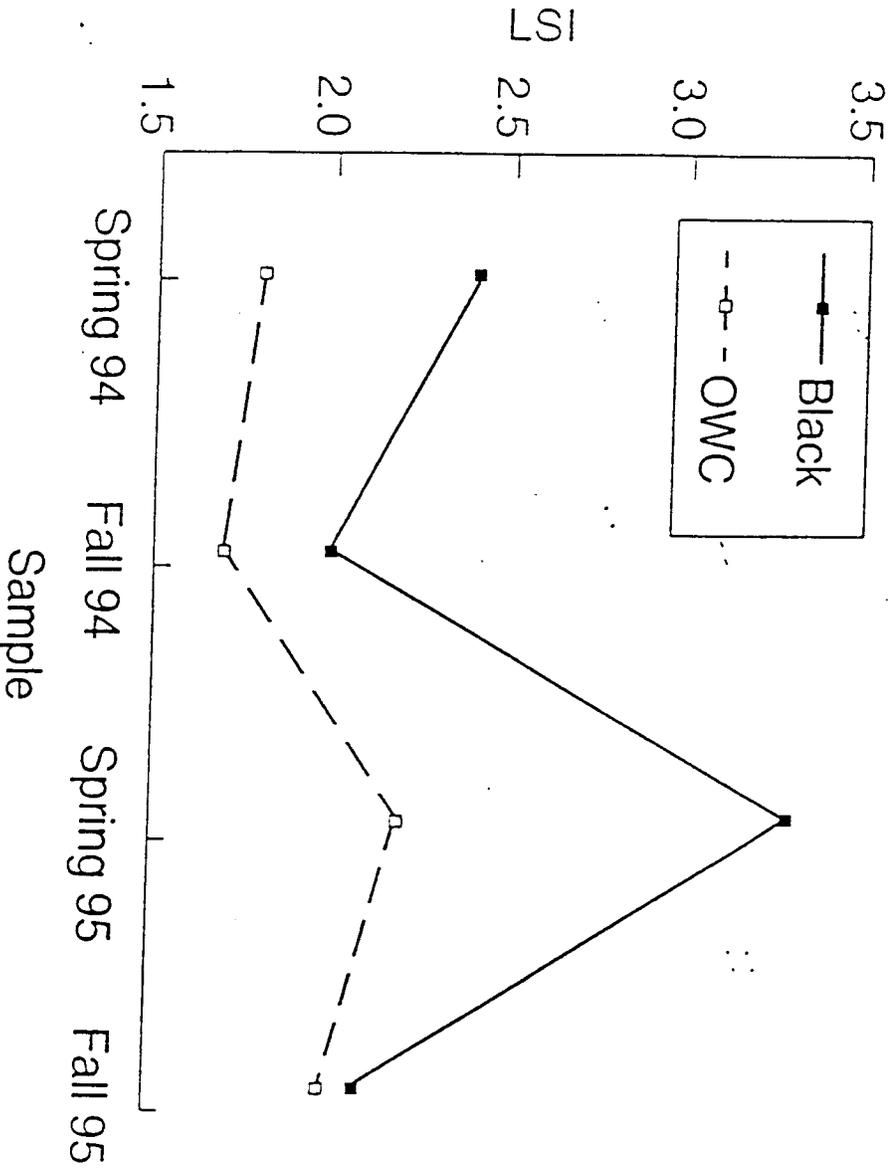


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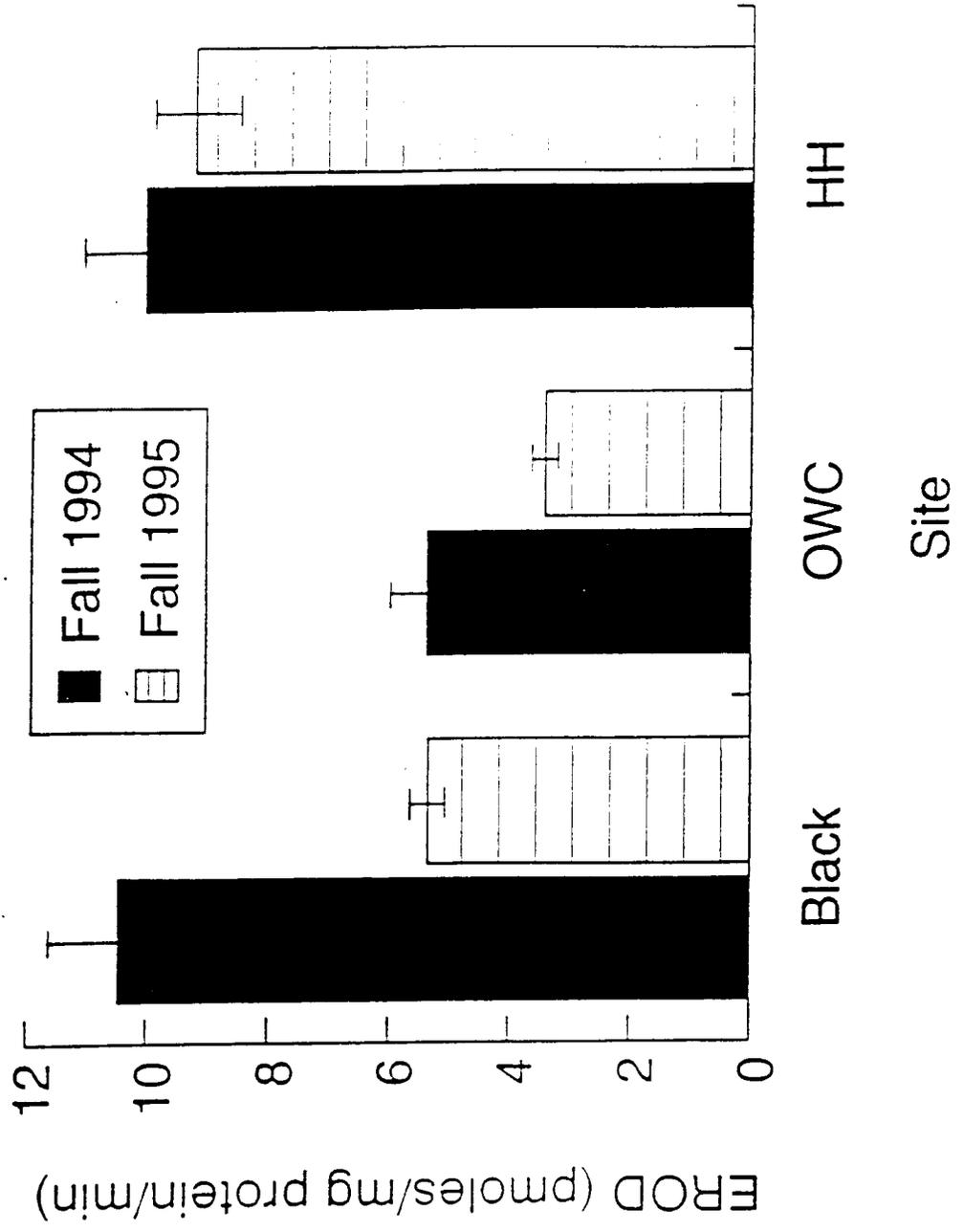


Figure 2.

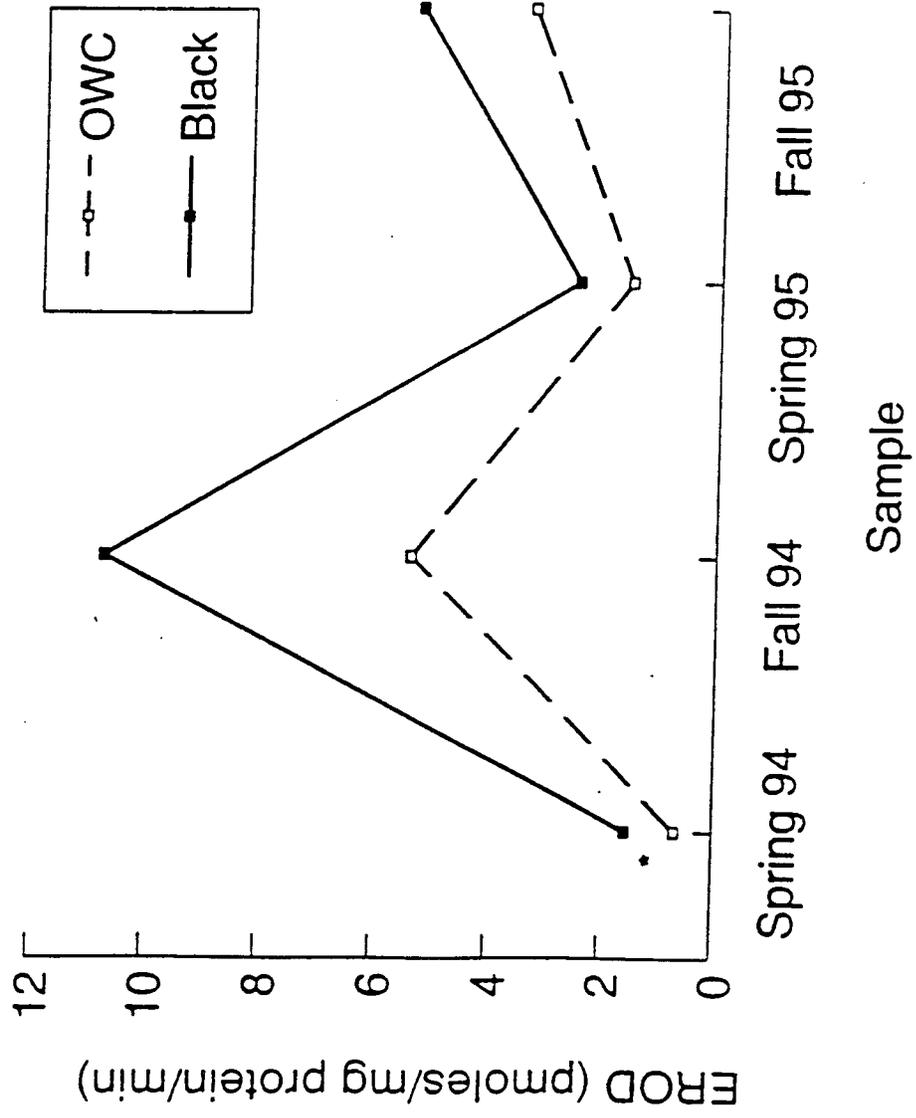
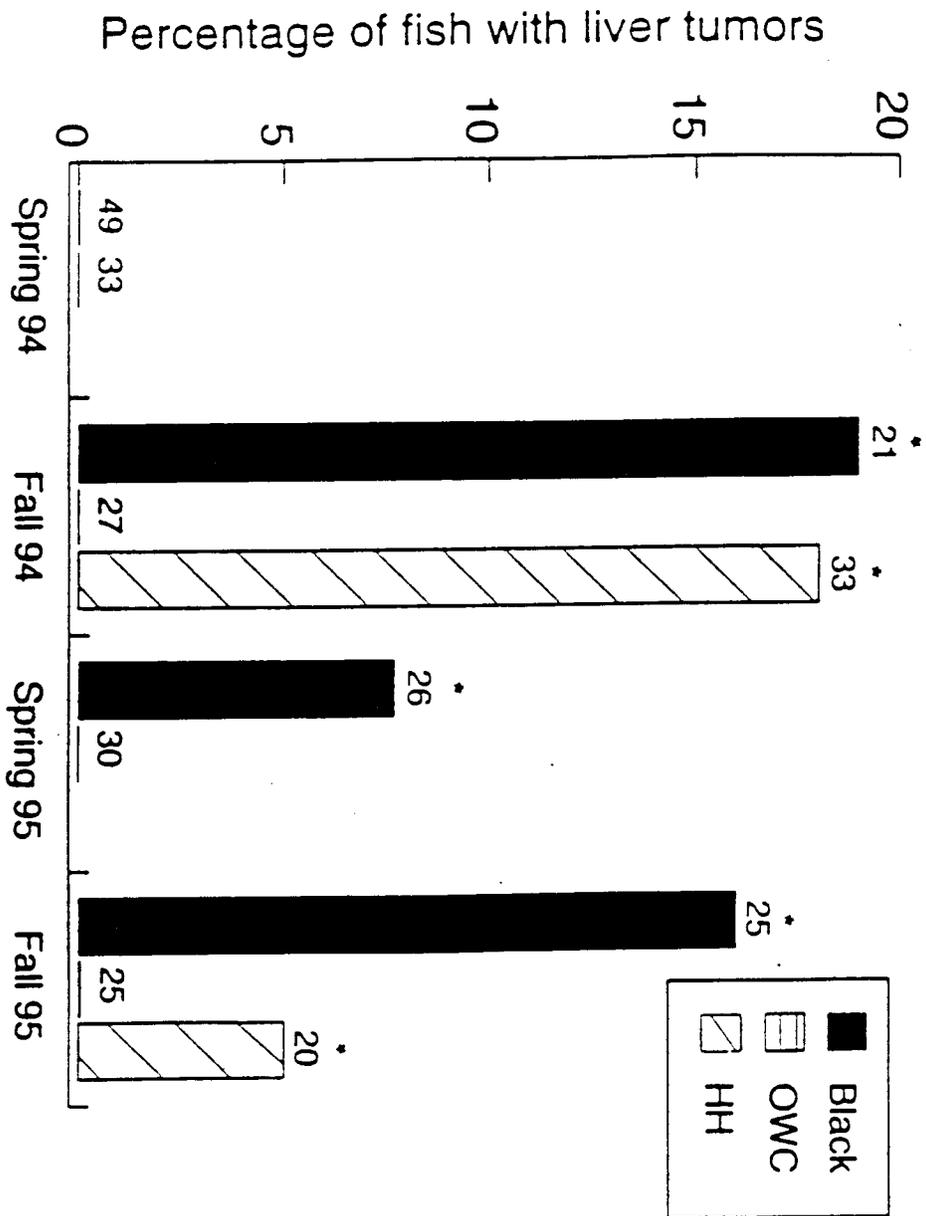


Figure 1.



## Figure Captions

**Figure 1.** The prevalence of grossly visible liver tumors in brown bullhead collected from the Black River, Old Woman Creek, and Hamilton Harbour. Sites within the same sampling time always appear in the order above. No collections were made in the spring from Hamilton Harbour. \*Indicates significantly greater than Old Woman Creek using chi-square at  $p < 0.05$ . Sample sizes are shown above each bar.

**Figure 2.** EROD activity in brown bullhead collected from the Black River and Old Woman Creek from the spring of 1994 to the fall of 1995. \*EROD values in the spring of 1994 are not significantly different using ANOVA at  $p < .05$ .

**Figure 3.** EROD activity in brown bullhead collected from the Black River, Hamilton Harbour, and Old Woman Creek in the fall of 1994 and 1995.

**Figure 4.** Liver Somatic Index (LSI) in brown bullhead collected from the Black River and Old Woman Creek from the spring of 1994 to the fall of 1995. \*LSI values in the fall of 1995 are not significantly different using ANOVA at  $p < 0.05$ .