Bull. Environ. Contam. Toxicol. (1995) 54:50–59

Environmental Contamination and Toxicology

Pathological and Serum Chemistry Profiles of Brown Bullheads (*Ameiurus nebulosus*) from the Black River and Old Woman Creek, Ohio

L. C. Folmar[1], J. Harshbarger[2], P. C. Baumann[3], G. Gardner[4], S. Bonomelli[5]

[1]U.S. Environmental Protection Agency, 1 Sabine Island Drive, Gulf Breeze, Florida 32561, USA
[2]Registry of Tumors in Lower Animals, MRC 163, Smithsonian Institution, Washington, D.C. 20560, USA
[3]U.S. Fish and Wildlife Service, 2021 Coffey Rd, Columbus, Ohio 43210, USA
[4]U.S. Environmental Protection Agency, 27 Tarzwell Drive, Narragansett, Rhode Island 02882, USA
[5]Biology Department, University of West Florida, 11000 University Parkway, Pensacola, Florida 32514, USA

Received: 11 November 1993/Accepted: 15 June 1994

During the past two decades there have been numerous reports concerning epizootics of neoplasia in feral fish (Baumann et al. 1990; Baumann 1992; Harshbarger and Clark 1990). One of those reviews (Harshbarger and Clark 1990) identified 41 locations with such epizootics in North America alone. While some neoplasms in fish are of viral origin, many investigations have linked both benign and malignant neoplasms to carcinogenic contaminants in the environment (Baumann et al. 1990; Harshbarger et al. 1993). Serum chemistry measurements have been made on fish exposed to a variety of xenobiotic chemicals in the laboratory and on fish collected from highly contaminated areas in the environment (Folmar 1993). There is, however, little information on relationships between serum chemistry and neoplastic lesions in feral fish (Casillas et al. 1985; Folmar et al. 1993b). The purpose of this investigation was to determine whether serum chemistry measurements could be used as predictive indices of neoplasia in tumor-bearing brown bullheads (Ameiurus nebulosus) collected from the Black River, Ohio.

METHODS AND MATERIALS

Brown bullheads were collected in June 1992, by overnight sets of fyke nets (12 or 25-mm mesh) in two Lake Erie tributaries: the Black River (BR) at Lorraine, Ohio and Old Woman Creek (OWC) at Huron, Ohio. Near the collection site, the Black River receives effluent from a steel plant and historically received effluent from an associated coking facility. Old Woman Creek, approximately 32 km west of the Black River, has a small, primarily agricultural watershed. Its lower reach, which includes the collection site, is a part of the Old Woman Creek National Estuarine Sanctuary.

Fish were initially sized to select fish of greater than 250 mm (age 2+) for serum analysis and histopathology,

Correspondence to: L. C. Folmar

since previous investigations have shown that less than 2% of the age 2 fish from this region showed grossly visible hepatic tumors (Baumann et al. 1987). The fish were anaesthetized with MS-222 and their blood drawn from the caudal vein using red top Vacutainers. The serum was removed and stored on ice until analyzed. Pectoral spines were removed and aged according to methods previously described (Baumann et al. 1990). The livers were excised and fixed in 10% buffered formalin within 1 hr of collection. Sagittal and parasagittal tissue blocks were made of each lobe and any grossly visible nodular lesions. Blocks were sectioned at 4 to 6 um, and stained with hematoxylin and eosin.

Serum samples were analyzed on a fully automated, computer-controlled, clinical chemistry analyzer (Beckman CX-5, LeBrea, CA). All test conditions and reagents were standard for the instrument and obtained from Beckman.

Sodium (Na), potassium (K), chloride (Cl) and carbon dioxide (CO_2) were measured by ion-specific electrodes at ambient temperature. Enzymatic rate determinations were used for alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LD-L), creatine kinase (CK) and blood urea nitrogen (BUN). Creatinine (Crea) was measured by the modified-rate Jaffe method. Albumin (Albg) was measured using bromcresol green reagent. The remaining assays for calcium (Ca), cholesterol (Chol), glucose (Glu), inorganic phosphorous (Pi), total protein (TP), triglycerides (Trig), uric acid (Uric), magnesium (Mg) and iron (Fe) were colorimetric determinations. All enzyme and colorimetric determinations were made at 37° C (Tietz 1986).

For the serum chemistry measurements, statistically significant differences between locations were determined by analysis of variance (ANOVA). Relationships among the various serum chemistry parameters or pathological observations were determined by Pearson's Product Moment Test of Correlation. Stepwise logistic regression analyses were used to determine predictive relationships between serum chemistry measurements and the histopathological observations (SAS 1990).

RESULTS AND DISCUSSION

Twenty-nine, tumor-free brown bullheads (17 male, 12 female) were selected from the fish collected from Old Woman Creek. Fourteen of the 29 fish (48%) were Age 3, 9 (31%) were Age 4, and 4 (14%) were Age 5. One fish was Age 2 and one was Age 6.
Twenty-six of the 46 fish (57%) collected from the Black River were female. Of those fish, 21 (46%) were Age 3,

12 (26%) were Age 4, 10 (22%) were Age 5 and 3 (7%) were Age 6. Histopathological evaluations showed that the incidence of neoplasia diagnosed as cancer was 52%.

There were 22 cases (48%) of biliary neoplasms; 3 cholangiomas, and 19 cholangiocellular carcinomas. The cholangiomas were identified as expanding masses of well-differentiated bile ducts which did not appear invasive. Cholangiocellular carcinomas showed atypical or poorly differentiated bile ducts with varying amounts of fibrous stroma interdigitating with normal liver. They ranged from minute to large (greater than 1.0 cm in diameter) with less evidence of a stepwise progression than the hepatocellular neoplasms. Larger lesions were often centrally necrotic (Figure 1).

Fourteen cases (30%) of hepatocellular carcinoma were also observed (Figure 2). These neoplasms showed enlarged hepatocytes distinguished by increased cytological atypia, pattern atypia and often interdigitated with the adjacent normal liver. These lesions ranged from 1.5 to 4.0 mm (Figure 3). Eleven (24%) of the fish showed both a biliary neoplasm and hepatocellular carcinoma.

Additionally, eight fish (17%) showed foci of cellular alterations in the liver. The foci were small clones of well-differentiated hepatocytes distinguished by differential staining. The median diameter of these foci was about 500 um, or about 50 cells. Liver cords within the lesion blended into adjacent normal liver cords without compression. It is unclear whether these alterations represent preneoplastic lesions or incipient neoplasms, but they are definitely in the sequence of tumor formation. Five fish showed external tumors; 4 squamous cell carcinomas and one epidermal papilloma. One case of presumptive mesothelioma was also observed.

Female bullheads had a higher percentage of both hepatic (79%) and biliary (55%) neoplasms than did males. The age class distribution of the tumors was as follows: 6 in Age 3; 7 in Age 4; 9 in Age 5 and 2 in age 6.

In previous studies (Baumann and Harshbarger 1985; Baumann et al. 1982; Baumann et al. 1987; Baumann et al. 1990) both hepatic and biliary tumors were observed in fish collected from the Black River at a site heavily contaminated with a variety of polynuclear aromatic hydrocarbons (PAHs), including benzo(a)pyrene (BaP) (Baumann and Harshbarger 1985; Baumann et al. 1987). The focus of this investigation was not to relate the observed lesions (pathologic abnormalities) or serum chemistry measurements to specific concentrations of xenobiotic chemicals, but to establish whether a

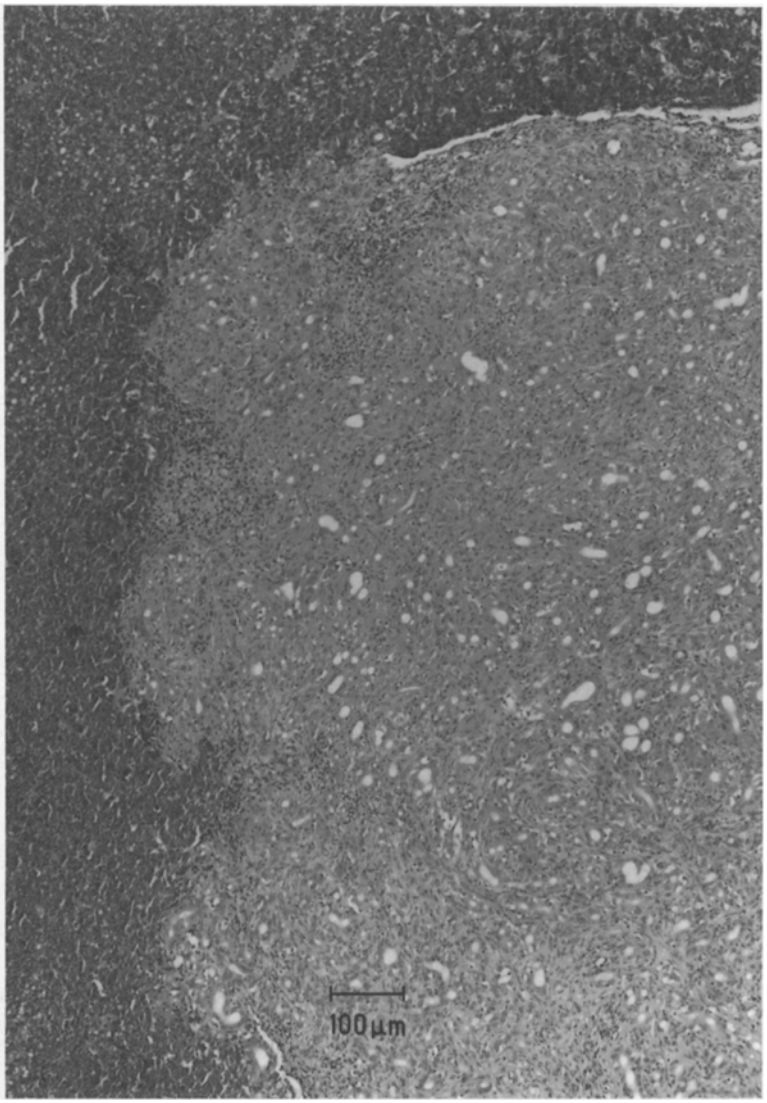

Figure 1. Cholangiocellular carcinoma. Edge of a 2.0 mm mass (top) invading darker normal liver (bottom). The empty spaces in the cancer are lumens of poorly formed bile ducts or ductules. The solid portions between the ductules consist of fibrous stroma.

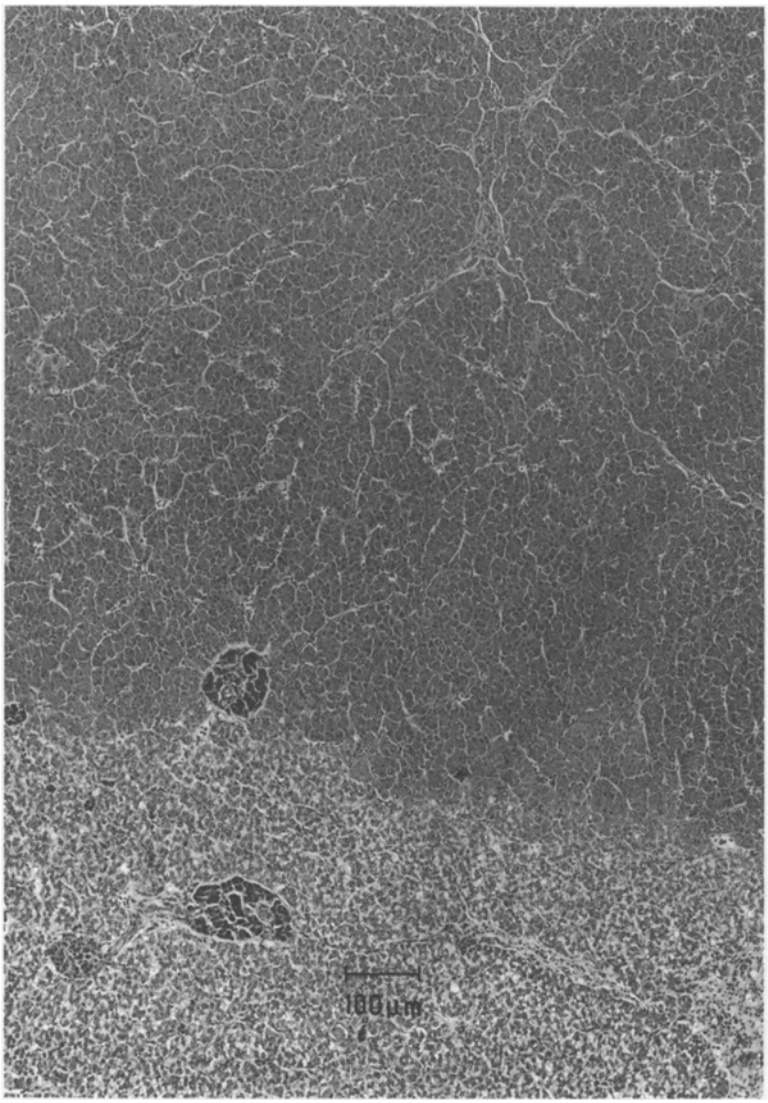

Figure 2. Hepatocellular carcinoma. Edge of a 4.0 mm, dense, basophilic mass (top) invading normal liver (bottom). Reduced cytoplasmic glycogen largely accounts for the denser appearance an enhanced chromophilia of the tumor mass. The liver cords are also thicker, thus producing a more solid pattern. The advancing lower left edge of the cancer is beginning to envelop a dark pancreatic acinus.

predictive relationship existed between significant pathological changes and serum chemistry measurements.

The prevalence of tumors increased from 30% in Age 3 fish to 67% in Age 4 and 90% in Age 5. Two of the three Age 6 fish collected had cancerous lesions. Although the sample size was small, these figures are consistent with the positively correlated age and tumor frequencies reported for bullheads caught in 1982 (Baumann et al. 1990). The total percentage of tumor-bearing fish was approximately the same in the 1992 sample (58%) as in 1982 (60%) (Baumann and Harshbarger 1985), and the ratio of cholangiocellular carcinomas to hepatocellular carcinomas (1.5:1) remained constant. The observation of a significantly greater incidence of hepatocellular, but not biliary cancer, in female fish also confirms our previous observation of brown bullheads collected in 1981-1982 (Baumann et al. 1990).

At least 29 (65%) of the bullheads from the Black River and 18 (62%) from Old Woman Creek had parasitic infections of Proteocephalus ambloplitis (Phylum Platyhelminthes, Class Cestoda). Twenty two (49%) of the Black River fish and 19 (65%) of the Old Woman Creek fish exhibited a bile duct infection of Myxidium mellum (Phylum Myxozoa, Class Myxosporea).

The interactions among parasites, natural environmental factors, anthropogenic factors and host organisms are poorly understood. Pollutants can increase susceptibility or reduce resistance in the host organism. These same chemicals can also eliminate or provide a food source for intermediate hosts (Overstreet 1993). Based upon an equal number of sections, the bullheads from Old Woman Creek were more heavily parasitized by M. mellum than were the fish from the Black River. This observation may reflect a contaminant effect on the preferred intermediate host for the myxosporean parasite. There were no differences in the prevalence of cestode infections between the two locations, suggesting that chemical contaminants did not directly affect the viability of the cestode, nor did they make the host more susceptible to infection.

The "reference" serum chemistry values from the Old Woman Creek fish were derived from the 29 fish which showed no hepatic or biliary tumors. Mean serum chemistry measurements for fish from Old Woman Creek and the Black River are presented in Table 1. Fish from the Black River location showed significantly greater serum ALT, AST, LDL, TP, BUN, triglycerides, calcium and significantly less serum phosphorus than did the fish from Old Woman Creek. Analyses of fish from both OWC and BR showed the following serum chemistries to be

Table 1. Mean serum chemistry values for fish collected from Old Woman Creek (OWC), and the Black River (BR).

Measure/ units		OWC	BR
Na	mmol/L	128 ± 3(29)	126.5 ± 6.5(45)
K	mmol/L	2.0 ± 0.7(29)	2.0 ± 0.8(45)
Cl	mmol/L	110 ± 3(29)	108.0 ± 7.9(45)
Crea	mg/dL	0.3 ± 0.2(29)	0.4 ± 0.2(45)
BUN	mg/dL	1.9 ± 0.4(29)	2.6 ± 0.8(45)*
Glu	mg/dL	141 ± 40(29)	133.3 ± 36.0(45)
TP	g/dL	2.9 ± 0.3(29)	3.9 ± 0.6(45)*
Ca	mg/dL	10.7 ± 1.2(29)	14.4 ± 6.0(45)*
AST	IU/L	358 ± 74(29)	673 ± 470(45)*
ALT	IU/L	4.0 ± 1.0(29)	8.0 ± 4.2(45)*
CK	IU/L	3277 ± 1808(29)	3350 ± 1727(43)
LDL	IU/L	282 ± 77(29)	437 ± 389(44)*
ALP	IU/L	10.6 ± 2.0(29)	12.7 ± 13.8(45)
Uric	mg/dL	1.3 ± 0.5(29)	1.2 ± 0.5(45)
Trig	mg/dL	46.1 ± 17.3(29)	114.6 ± 71(45)*
Phos	mg/dL	16.1 ± 3.1(29)	12.9 ± 2.5(45)*
Chol	mg/dL	128 ± 42(29)	134.8 ± 39.8(45)
Iron	mg/dL	61 ± 25(29)	61.5 ± 24.2(45)
Mg	mg/dL	4.6 ± 0.5(29)	3.7 ± 0.6(45)
Albg	g/dL	1.4 + 0.2(29)	1.4 + 0.2(45)

Number of samples in parentheses. (* = significantly different at $P \leq 0.05$)

significantly correlated with one another: Na, Cl, and Albg; TP and Albg; AST and ALT; Ca, Trig, Phos, Chol, and Mg. Additionally, in the fish from the Black River, the concentrations of the serum enzymes ALP, ALT, AST and LDL were all correlated with one another.

The significantly elevated concentrations of serum ALT, AST and LDL (Table 1) are all consistent with liver parenchymal damage, at least in humans and other mammals (Tietz 1986). Increases in serum total protein concentrations may reflect increases in liver enzymes and other tumor-specific proteins (Denslow et al. 1994). Serum triglycerides can increase due to liver damage and as a mechanism to sequester lipophilic xenobiotics (Tietz 1986). Increased serum BUN concentrations are commonly associated with kidney pathology in mammals (Tietz 1986); however, we made no histopathological evaluations of the kidneys of these fish to investigate the possibility of renal disease. Increased serum calcium and decreased serum phosphate concentrations may have resulted from tumor secreted estrogen-stimulated vitellogenesis (Folmar et al. 1994). Total serum estrogen (estrone plus estradiol) was significantly elevated in both males and post-vitellogenic females with tumors. Significant correlations between serum Na and Cl have been demonstrated for many species of fish (Folmar and Dickhoff 1980). Also, significant correlations between serum (TP and Albg), (AST and ALT), (Ca, Trig, Phos, and Chol) have been previously demonstrated in striped mullet (Mugil cephalus) and pinfish (Lagodon rhomboides) (Folmar et al. 1992; 1993a).

All of the serum chemistry measurements were compared with the presence/absence of each pathologic abnormality and parasitosis in each individual fish. Although the means of the serum chemistry measurements from the Black River location clearly indicated liver abnormalities (Table 1), no serum measurement(s) was found to be predictive of any single neoplasm (cholangioma, cholangiocellular carcinoma, hepatocellular carcinoma), focus of hepatocellular alteration, or the presence of a liver parasite. The absence of significant predictable relationships within the individual fish possibly reflects the small sample sizes (3 fish with cholangiomas, 19 fish with cholangiocellular carcinomas and 14 with hepatocellular carcinomas), natural variability (Folmar et al. 1992) and variable physiological responses to the presence of the tumors. Perhaps with continued evaluations of serum chemistry parameters from tumor-bearing fish, statistically significant relationships will emerge.

Acknowledgments. This research was supported by a Cooperative Research Agreements between the U.S. E P A, Ecological Risk Assessment Program and the University of West Florida (CR-816731) and the U.S. Fish and Wildlife Service. The authors wish to thank Dr. J. Heltshe for statistical assistance and Dr. R. Overstreet for parasite identification.

REFERENCES

Baumann PC (1992) The use of tumors in wild populations of fish to assess ecosystem health. J Aquat Ecosyst Health 1:135-146

Baumann PC, Harshbarger JC (1985) Frequencies of liver neoplasia in a feral fish population and associated carcinogens. Mar Environ Res 17:324-327

Baumann PC, Smith WD, Ribick M (1982) Hepatic tumor rates and polynuclear aromatic hydrocarbon levels in two populations of brown bullheads (Ictalurus nebulosus) In: Cooke M, Dennis AJ, Fisher GL (eds.). Polynuclear Aromatic Hydrocarbons: Sixth International Symposium on Physical and Biological Chemistry. Battelle Press, Columbus, OH. pp 93-102

Baumann PC, Smith WD, Parland WK (1987) Tumor frequencies and contaminant concentrations in brown bullheads from an industrialized river and a recreational lake. Trans Amer Fish Soc 116:79-86

Baumann PC, Harshbarger JC, Hartman KJ (1990) Relationship between liver tumors and age in brown bullhead populations from two Lake Erie tributaries. Sci Total Environ 94:71-87

Casillas E, Myers MS, Rhodes LD, McCain BB (1985) Serum chemistry of diseased English sole, Parophrys vetulus Girard, from polluted areas of Puget Sound, Washington. J Fish Dis 8:437-449

Denslow ND, Chow MM, Folmar LC (1994) Isoforms of Apolipoprotein A-I in the serum of brown bullheads (Ameiurus nebulosus) with liver cancer. Can J Zool (In press)

Folmar LC (1993) Effects of chemical contaminants on the blood chemistry of teleost fish: a bibliography and synopsis of selected effects. Environ Toxicol Chem 12(2): 337-375

Folmar LC, Dickhoff WW (1980) The parr-smolt transformation (smoltification) and seawater adaptation in salmonids. A review of selected literature. Aquaculture 21:1-39

Folmar LC, Bonomelli S, Moody T, Gibson J (1992) Annual cycle of blood chemistry measurements in striped mullet (Mugil cephalus) and pinfish (Lagodon rhomboides) from the Gulf of Mexico. J Fish Biol 41:999-1011

Folmar LC, Moody T, Bonomelli S, Gibson J (1993a) The effect of short-term exposure to three chemicals on the blood chemistry of the pinfish (Lagodon rhomboides). Arch Environ Contam Toxicol 24:83-86

Folmar LC, Gardner G, Hickey J, Bonomelli S, Moody T (1993b) Serum chemistry and histopathological evaluations of brown bullheads (Ameiurus nebulosus) from the Buffalo and Niagara Rivers. Arch Environ Contam Toxicol 25:298-303

Folmar LC, Denslow ND, Wallace RA, LeFleur G, Bonomelli S. and Sullivan CV (1994) A highly conserved N-terminal sequence for teleost vitellogenin with potential value to the biochemistry, molecular biology and pathology of vitellogenesis. J Fish Biol (In press).

Harshbarger JC, Clark JB (1990) Epizootiology of neoplasms in bony fish of North America. Sci Total Environ 94:1-32

Harshbarger JC, Spero PM, Wolcott NM (1993) Neoplasms in wild fish from the marine ecosystem emphasizing environmental interactions. In: Couch JA, Fournie JW (eds) Pathobiology of Marine and Estuarine Organisms. CRC Press, Boca Raton, Florida, pp 157-176

Overstreet RM (1993) Parasitic diseases of fishes and their relationship with toxicants and other environmental factors. In: Couch JA, Fournie JW (eds) Pathobiology of Marine and Estuarine Organisms. CRC Press, Boca Raton, Florida, pp 111-156

SAS Institute, Inc (1990) SAS/GRAPH Software, Version 6, 1st Edition. Cary, N.C. SAS Institute, Inc.

Tietz N (ed) 1986. Textbook of Clinical Chemistry. W B Saunders Co, Philadelphia, PA 1919 pp