PIGMENTED SKIN TUMORS IN GIZZARD SHAD (*Dorosoma cepedianum*) FROM THE SOUTH CENTRAL UNITED STATES: RANGE EXTENSION AND FURTHER ETIOLOGICAL STUDIES

Gary K. Ostrander*§, David R. Geter,* William E. Hawkins,† Jay C. Means,‡

*Environmental Toxicology Program, Department of Zoology, Oklahoma State University, Stillwater, OK 74078, †Gulf Coast Research Laboratory, Institute of Marine Sciences, University of Southern Mississippi, Ocean Springs, MS 39566-7000, ‡Department of Veterinary Pharmacology and Toxicology, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803, § Department of Biology and Department of Comparative Medicine, The Johns Hopkins University, Baltimore, MD 21218-2685.

Address correspondence to:

Gary K. Ostrander
224 Mergenthaler Hall
3400 N. Charles Street
Johns Hopkins University
Baltimore, MD 21218-2685

TEL: (410) 516-8215
FAX: (410) 516-6017
E-mail: gofish@jhu.edu

Abstract-- Previous studies reported skin tumors diagnosed as pigmented subcutaneous spindle cell neoplasms in 22% of gizzard shad (*Dorosoma cepedianum*) from Lake of the Arbuckles, Oklahoma. Those studies could not confirm chemical carcinogens or retroviruses as etiological agents. The present study reports the neoplasms in 20% of shad from two additional lakes, Murray and Texoma in south-central Oklahoma, extending the range of the lesion. No neoplasms were found in shad from a reference site, Lake Carl Blackwell, OK. Further investigations into the etiology of the lesions were conducted. Significant levels of potentially carcinogenic trace elements in the water, sediment, or tissues were not identified by inductively coupled plasma mass spectrometry. Radioactivity analyzed by liquid scintillation counting of radon and gross alpha/beta radiation was not above background. Genetic marker and band sharing analysis by random amplified polymorphic DNA and double-stringency polymerase chain reaction could not separate tumor-bearing shad from nontumor-bearing ones. Of 2128 shad examined, 387 exhibited lesions with a significantly higher number occurring dorsally (79.5%) than ventrally (20.5%). Overall, this study showed the epizootic is not limited to a single lake and tended to rule out some known carcinogens and radioactivity as proximate causes for the epizootic.

INTRODUCTION

The etiology of neoplasia in wild fish generally varies with species and geographic site. Chemical contamination and oncogenic viruses are known causes of fish neoplasia [1]. Neoplastic lesions have been demonstrated to affect nearly every cell and tissue type in fishes from freshwater [2], estuarine, and marine habitats [3]. Neoplasia of the liver of fishes is usually considered to be caused by exposure to carcinogenic chemicals, particularly polynuclear aromatic hydrocarbons in sediments whereas non-hepatic neoplasia is rarer than hepatic neoplasia and is not as clearly related to environmental contamination [1,3]. Epizootics of non-hepatic neoplasia that have been associated with environmental contamination in the Great Lakes region include epidermal neoplasms in brown bullhead [4,5] and white suckers [6], dermal pigment cell neoplasms (chromatophoromas) and neurilemmomas in freshwater drum (*Aplodinotus grunniens*) [7], gonadal neoplasms in carp/goldfish hybrids [8,9,10], and several types of non-hepatic as well as hepatic neoplasms in sauger (*Stizostedion canadense*) and walleye (*S. vitreum*) [11]. Several epizootics of neoplasia appear to have a viral origin. These include lymphoma in the northern pike (*Esox lucius*) [12], plasmacytoid leukemia in chinook salmon (*Oncorhynchus tschawytscha*) [13], dermal sarcoma in walleye [14], and neurofibromatosis in bicolor damselfish (*Pomacentrus partitus*) [15].

The present study concerns an epizootic of pigmented subcutaneous spindle cell neoplasms that has been previously reported from gizzard shad (*Dorosoma cepedianum*) from Lake of the Arbuckles, a man-made lake in central Oklahoma, USA [16,17]. In these studies were reported lesions in about 22% of adult gizzard shad and 0% in nearly 2000 juvenile shad examined, and the occurrence of lesions did not appear to be seasonal. Likewise, there were no

differences in tumor incidence between adult males and females. Transmissibility studies conducted with rainbow trout injected with cell-free extracts of gizzard shad neoplasm were negative [17]. Ostrander et al. (17) described the appearance, distribution, and histology of the shad tumors. Grossly, the tumors were primarily distributed over the head, trunk, and fins as superficial raised masses that were usually darkly pigmented but sometimes unpigmented. Histologically, the tumors were located in the dermis, had a variable amount of connective tissue, and consisted of cells in a variety of forms and arrangements. Most tumors were composed of fusiform or spindle cells arranged in wavy bundles, whirling patterns or interwoven fascicles. Pigmentation was attributed to large dense deposits of melanin or to scattered individual melanin-containing cells. Immunohistochemical detection of proliferating cell nuclear antigen revealed a high proliferative activity in the spindle cells. Electron microscopy showed that the tumors were composed of several cell types, including host reactive cells, melanocytes in different stages of maturity, and fibroblast-like cells. The cell of origin of the poorly differentiated neoplasms was not determined but appeared to be neural, probably a pigment cell or a nerve sheath cell.

Fractionation of lake water and sediment samples followed by GC-MS analysis revealed no carcinogenic compounds when compared to the spectra in the NIST database [18] in which more than 42,000 compounds are archived. Assays for reverse transcriptase indicating the presence of retrovirus in tumor homogenates were negative and no evidence of viral particles were found in specimens examined by transmission electron microscopy [16]. Since those studies were published, additional cases of poorly differentiated dermal neoplasms have been found in other fish species from Lake of the Arbuckles including a hemangiopericytoma from a white bass (*Morone chrysops*) [19], and poorly differentiated spindle cell neoplasms from two

threadfin shad (*Dorosoma petenense*) [Geter et al., in prep.].

In the present study, we attempted to determine: (1) whether gizzard shad from lakes sharing the same drainage as Lake of the Arbuckles were affected by the neoplasms; (2) if potentially carcinogenic trace elements such as beryllium, chromium, nickel, arsenic, selenium, cadmium, mercury, and lead, detectable by inductively coupled plasma mass spectrometry (ICP-MS), were present in sediment, water, or tissues; (3) whether naturally occurring uranium deposits within the Lake of the Arbuckles and Lake Texoma watersheds contribute significant radioactivity to the study sites; (4) if genetic markers produced by random amplified polymorphic DNA (RAPD) and double-stringency polymerase chain reaction (DS PCR) could distinguish tumor-bearing from nontumor-bearing gizzard shad; and (5) whether the anatomic distribution of the tumors might provide a clue to their etiology.

MATERIALS AND METHODS

Materials

Unless noted otherwise, analytical grade reagents (Sigma Chemical Company, St. Louis, MO, USA) were used.

Fish collection

Gizzard shad were collected from Lake of the Arbuckles, Lake Texoma, Lake Murray, and Lake Carl Blackwell, (Figure 1), the reference site, either by beach seine (18 m, 1 cm mesh)

for gizzard shad (<1 year old) or by gill netting (100-m, 6 cm mesh) for mature gizzard shad (2-5 years old). Sites sampled in Lake Texoma were chosen to test for spatial tumor frequencies in the northern (Glasses), central (Caney), and western (Lebannon) areas of the lake. Adult gizzard shad (310 to 490 mm in length) were estimated to be 2 to 5 years old by standard-weight curves [20]. Nets were set perpendicular to the water flow and examined every 6 hours for 24-72 hours. Netted gizzard shad were weighed, measured, and examined grossly for tumor occurrence. Liver and muscle tissue were excised for inductively coupled plasma mass spectrometry (ICP-MS) analysis and liver tissue excised for both random amplified polymorphic DNA (RAPD) and double-stringency polymerase chain reaction (DS PCR) analysis.

Inductively coupled plasma mass spectrometry (ICP-MS)

ICP-MS analysis was conducted on sediment, water, and shad tissues (liver and muscle) from Lake of the Arbuckles and Lake Texoma to determine the presence of potentially carcinogenic trace elements (beryllium, chromium, nickel, arsenic, selenium, cadmium, mercury, and lead)[21]. Sediment samples were obtained with an Ekman dredge, hypolimnetic water samples with a Van Dorn sampler, and epilimnetic water samples by hand 5 cm below the water surface. Sediment and water samples were placed in 50 ml acid-washed polyethylene tubes (Fisher Scientific Co, Pittsburgh, PA). Samples and blanks were placed on ice, transported to the laboratory in darkness, and stored at 4°C for two days in darkness before shipping. Water samples and trip blanks were filtered with Whatman glass microfibre filters. Filtered water and filter were placed in separate precleaned, acid washed 100 ml glass sample vials that were wrapped in aluminum. About 1.0-1.5 g (wet wt) liver and muscle were excised from six tumor-

bearing and six nontumor-bearing gizzard shad from both Lake of the Arbuckles and Lake Texoma. Dissected tissues were placed in sterile 2 ml centrifuge tubes, placed on dry ice for transport to the laboratory, and then stored at -20°C. Sediment and water samples were shipped on ice, and tissue samples were shipped on dry ice for ICP-MS analysis.

All reagents and chemicals utilized in these procedures were ultra-pure grade to minimize the introduction of metals. National Institute of Standards and Technology's Standard Reference Materials (SRM) 1646 Estuarine Sediment and SRM 1635 Oyster Tissue were analyzed in parallel with each set of samples to control for contamination and define recovery. Techniques for sample processing, acid digestion, and trace element solubilization were conducted to maximize recovery of *in situ* trace elements, and to minimize or exclude extraneous metal contamination using a modification of Environmental Protection Agency Method 6020. All steps in which contamination could be extraneously introduced were carried out under a level 100 laminar flow hood. Water samples were analyzed following a 1 to 10 dilution in 3% ultra-pure nitric acid solution and samples were analyzed within one week of arrival.

Sediment and tissue samples were thawed and the entire sample transferred to a metal-free vessel for thorough homogenization. An aliquot of the homogenate was transferred to a metal free polypropylene digestion vessel for digestion using a CEM 2000 (CEM Corp., Matthews, NC) microwave digestion system. Digestions were performed using approximately 1 g (wet wt) sediment or tissue in 10 ml 50% ultra-pure nitric acid for approximately two hours. Samples were diluted to 50 ml final volume and a 1 ml aliquot was analyzed for trace element concentrations. Samples of MilliQ water and reagent acids were retained for trace element determinations as blanks or reagent blanks with each set of digest. Yttrium 89 was used as an internal standard in all samples.

Analyses of elements were performed on each sample using a Fisons PlasmaQuad II+ ICP-MS. Samples were analyzed using triplicate, one minute data acquisition/integration times. Final trace element concentrations were blank subtracted and corrected for internal standard recovery, analysis dilution, digestion volume, and the original mass of the sample.

Environmental radiation

Water samples for gross alpha/beta and radon-222 radiation analysis were taken from the same locations as water samples for ICP-MS. Alpha/beta radiation samples were treated and analyzed by a modified procedure from Sanchez-Cabeza and Pujol [22] using a Packard Instruments 2770 TR/SL (time-resolved/ super low level) scintillation counter equipped with pulse decay discrimination (PDD) circuitry which separates alpha from beta events.

Random amplified polymorphic DNA (RAPD) and double-stringency polymerase chain reaction (DS PCR) analysis

Tumor-bearing and nontumor-bearing gizzard shad from Lake of the Arbuckles were weighed, measured, and livers excised. Dissecting scissors were soaked in 100% ethanol and thoroughly cleaned prior to each dissection. Tissues were individually wrapped in 30 x 30 cm sheets of autoclaved aluminum foil, placed in plastic freezer bags, and kept on dry ice until they were brought to the laboratory, and stored at -20°C. DNA extraction was accomplished by standard phenol/chloroform separation followed by ethanol precipitation and stored in a Tris EDTA (TE) buffer at 4°C [23]. For this study, we used both RAPD and DS PCR techniques to

produce genetic markers. Double-stringency polymerase chain reaction mixtures used two primers with different annealing temperatures [24]. The first primer, a M13 (CTCCCACCRCCRAGT) core microsatellite primer (Oklahoma State University Recombinant DNA/Protein Resource Facility, Stillwater, OK) amplifies a region between microsatellites, whereas the second primer, a standard 10-mer RAPD primer (Operon Technologies Inc., Alameda, CA) amplifies the products of the first primer. For analysis we used the 'Kit B' set of RAPD primers, which contained 20 individual sequences. The DS PCR reactions were carried out using a modified procedure from Matioli and deBrito [24] and RAPD reactions were performed using a modified procedure from Lynch and Milligan [25].

The PCR products were electrophoresed in 1 X TBE (9 mM Tris-borate, 0.2 mM EDTA, pH 8.0) at 25 V for 5 h in 5.0% polyacrylamide in 1 X TBE, [23], stained with ethidium bromide, examined under UV light, and photographed. Eight individuals were used for genetic marker comparisons, four tumor bearing (lanes 1-4) and four nontumor bearing (lanes 5-8). Lane 9 contained 1μg of 100 bp size standard (Cat. # 15628-050, Gibco-BRL, Gaithersburg, MD) used as a size reference during visualization and scoring.

Bands were initially observed for marker differences between tumor-bearing and nontumor-bearing gizzard shad and were then hand scored according to migration distance and incorporated into a presence-absence matrix. From this matrix, a band-sharing index (BS) was calculated as $BS = 2 N_{ab}/(N_a + N_b)$, where N_{ab} is the number of shared bands, N_a is the number of bands in one lane, and N_b is the number of bands in the other lane [26]. Band sharing indices were calculated for tumor bearing (lanes 1-4), nontumor bearing (lanes 5-8), tumor bearing vs. non-tumor-bearing (lanes 1-4 vs. 5-8), and a total comparison of all individuals (lanes 1-8). All individuals scored were present on the same gel for a total of 40 gels (20 RAPD and 20 DS

PCR).

Tumor location

The anatomical location of grossly visible tumors was noted and analyzed statistically to determine whether a pattern emerged. We considered all grossly visible tumors to be neoplastic lesions because we have not histologically determined the progression of the lesions. That is, small tumors can appear as pathologically advanced as large tumors. These fish have been extensively sampled over the last 7 years. Extensive analyses of all tumors were conducted initially. After looking at over 1,000 shad it became apparent that tumors could be identified by macroscopic examination. Nonetheless, anytime there is a question; the tissues are subject to complete histopathological examination. To systematically record tumor location, a schematic diagram of a gizzard shad was produced which was divided into dorsal and ventral sections by a horizontal line from the opening of the mouth to the middle of the caudal fin. The dorsal and ventral sections were then divided into three sections by vertical lines running down from the anterior base of the dorsal fin and the base of the caudal fin. The dorsal fin was included in the postero-dorsal section and all ventral fins were included in the postero-ventral section to assess fin tumor occurrence.

Statistics

Fisher's exact test was used to determine differences between Lake Texoma and Lake of the Arbuckles for neoplasm occurrence in the gizzard shad population. A paired T-test was used

to determine if differences existed between the prior studies and the present studies with regard to neoplasm occurrence in shad from Lake of the Arbuckles and was also used to test for differences between male and female tumor occurrence.

Concentrations for ICP-MS were reported as μg/L for water samples and μg/g wet weight for sediment, liver, and muscle tissues. Concentrations below the detection limits of the ICP-MS were represented as zero for statistical analysis. For analysis of the concentrations of trace elements (beryllium, chromium, nickel, arsenic, selenium, cadmium, mercury, and lead), all data were initially tested for normality and homogeneity of variance. Trace element concentrations for the liver and muscle tissues were not normally distributed, and appropriate nonparametric statistics were performed. A one-way Wilcoxon signed ranks two sample test was performed on the data from the liver and muscle tissues.

Paired T-tests were conducted on the RAPD and DS PCR band sharing data to determine whether differences existed between tumor-bearing and nontumor-bearing gizzard shad.

A one way ANOVA was performed to distinguish differences from the tumor location data area matrix and a paired T-test applied to test differences between dorsal and ventral locations. All statistical analyses were performed at the $p = 0.05$ level of significance with the Statistical Analysis Software from SAS Institute, Cary, NC.

RESULTS

Neoplasm prevalence

Gizzard shad collected from the Lake of the Arbuckles had a total neoplasm prevalence of 15.4% (73/474) which was lower than that reported in previous studies, 21.01% (208/990) [16,17] (Table 1). However, statistical testing for differences between previous studies and the current study failed to reveal a difference in neoplasm prevalence ($p = 0.469$). Analysis of Lake of the Arbuckles male and female gizzard shad (N = 51) showed no significant differences between the sexes in tumor occurrence ($p = 0.325$). Collections from Lakes Texoma and Murray showed neoplasm prevalences of 16.8% (111/660) and 20% (4/20), respectively. No significant difference was noted in neoplasm prevalence between Lake of the Arbuckles and Lakes Texoma ($p = 0.213$). Also, neoplasm occurrence was rather evenly distributed among collection sites in Lake Texoma including the Glasses (N = 81, 14.8%), Caney (N = 16, 12.5%), and Lebannon (N = 498, 17.1%) sites. Gizzard shad (N = 44 adults, N = 200 juveniles, from all studies) collected from Lake Carl Blackwell did not exhibit grossly observable neoplasms.

Inductively coupled plasma mass spectroscopy (ICP-MS)

ICP-MS analyses for beryllium, chromium, nickel, arsenic, selenium, cadmium, mercury, and lead were conducted on sediment, water, and shad tissues (liver and muscle) from Lake of the Arbuckles and Lake Texoma. Detectable levels of trace elements in the sediments and water were below U.S. EPA guideline maximum values for both lakes. Analysis of tumor-

bearing and nontumor-bearing tissues showed statistical differences between beryllium (<0.05 vs. 0.79 μg/g) and nickel (<0.05 vs. 21.25 μg/g) in the liver and nickel (10.35 vs. 4.48 μg/g) in the muscle.

Environmental radiation

Radioactivity levels in 45 water samples from Lake of the Arbuckles, Lake Texoma, and Lake Carl Blackwell ranged from <0.07-0.51 Bq liter $^{-1}$ for alpha, <0.40-1.60 Bq liter $^{-1}$ for beta, and <100 pCi liter $^{-1}$ for radon 222 radiation. All samples were well below U.S. EPA guidelines for alpha, beta, and radon radiation [27,28].

Random amplified polymorphic DNA (RAPD) and double-stringency polymerase chain reaction (DS PCR) analysis

Tumor-bearing gizzard shad were indistinguishable from nontumor-bearing gizzard shad by visible genetic marker comparison. Band sharing analysis also showed no difference between the tumor-bearing and nontumor-bearing gizzard shad with RAPD (p = 0.294) or DS PCR (p = 0.236) markers.

Tumor location

The location of 577 tumors from 346 tumor-bearing gizzard shad (Table 2) from Lake of the Arbuckles and Lake Texoma were scored. The occurrence of tumors in the dorsal section

(459/577=79.5%) was significantly higher than that in the ventral section (118/577=20.5%), (p = 0.001). Lesions were particularly abundant in the anterior-dorsal portion of the fish with 42.3% (244/577) occurrence (p = 0.001). Of the 229 tumor-bearing gizzard shad that exhibited a single tumor, 44.5% (102/229) had the tumor in the anterior-dorsal section, and 82.1% (188/229) had the tumor in one of the three dorsal sections. About one-third of the tumor-bearing gizzard shad had multiple tumors (117 of 346). Of those 117 specimens with multiple tumors, 86 (73.5%) had at least one tumor in the anterior-dorsal section, and 98.3% (115/117) had at least one tumor in one of the three dorsal sections.

DISCUSSION

An epizootic of pigmented subcutaneous spindle cell neoplasms in gizzard shad (*Dorosoma cepedianum*) was first observed in 1991 and was thought to be limited to Lake of the Arbuckles, Oklahoma. The epizootic has now been documented in two additional lakes, Texoma and Murray, which are about 55 kilometers south of Lake of the Arbuckles but share drainages. The reference site, Lake Carl Blackwell, is 180 kilometers north and lies in a different drainage and remains free of tumor bearing fish. The purpose of the paper was not to define, with the highest level of confidence the tumor incidence in these additional lakes other lakes. Instead, it was to report that unusual tumors are found at other locations in the drainage basin.. Neither the geographical extent nor when the date the epizootic actually began are presently known. Both Lake of the Arbuckles and Lake Murray were stocked by the Oklahoma Department of Water Quality with gizzard shad and threadfin shad (*D. pentenense*) from Lake Texoma in 1980 to provide forage for game fish [J. Pigg, Oklahoma State Department of Health, personal

communication]. This suggests that antecedents of tumor-bearing shad from Lake of the Arbuckles and Lake Murray were introduced from the same source at the same time. Department of Water Quality records for Lake Texoma show shad species occurring there naturally [J. Pigg, personal communication].

At sites of the gizzard shad neoplasm epizootic, the presence of trace elements (beryllium, chromium, nickel, arsenic, selenium, cadmium, mercury, and lead) that might play some role in carcinogenesis was determined by ICP-MS, which detects exceptionally low levels of most elements in the periodic table [29]. The technique has also been used for simultaneous analysis of multiple elements in biological materials [30,31]. ICP-MS analysis of the sediment and water samples revealed trace element concentrations below suggested levels for both the U.S. EPA and the State of Oklahoma [32,33]. In addition, comparison of tested trace elements in gizzard shad tissue to levels in other teleosts species showed shad to be within average concentrations [34]. Although we cannot account for the differences in concentrations of beryllium and nickel in tumor-bearing versus non-tumor-bearing shad, the available data suggest that those or other trace elements are not involved in the development of the shad neoplasms because of their low concentrations.

Because naturally occurring radiation can harm aquatic systems by producing a range of syndromes from reduced vigor to lethality, shortened life span, diminished reproductive rate, and genetic transmission of radiation-altered genes [35], we investigated whether background radiation might be a cause or contribute to the gizzard shad neoplasia. An Oklahoma Geological Survey minerals map published in 1969 showed deposits of uranium within the watershed around Lake of the Arbuckles and Lake Texoma. The uranium was mostly disseminated in gray sandstone and gray to black shales, and occurred in small low grade deposits ranging from 0.2-70

ppm uranium. Also, local deposits of crude oil and asphalt contained higher than normal amounts of uranium [36,37,38]. Evaluation of environmental alpha/beta radiation and radon-222 levels in the watersheds of both Lake of the Arbuckles and Lake Texoma revealed values below U.S. EPA drinking water guidelines [27,28], suggesting that radiation probably is not a factor in gizzard shad neoplasms.

Random amplified polymorphic DNA (RAPD) and double-stringency polymerase chain reaction (DS PCR) were used in an effort to generate a genetic marker to identify and separate tumor-bearing and nontumor-bearing gizzard shad. RAPD analysis has been used for genetic mapping, plant and animal breeding applications, and population genetics [39]. We analyzed variation between the tumor-bearing and nontumor-bearing gizzard shad first by visual comparison in attempts to find a "marker" to separate the two, then as a binary (presence/absence) value. With RAPD and DS PCR analysis, a genetic marker was not identified and no statistical difference was noticed in the band sharing values between tumor-bearing and nontumor-bearing gizzard shad using both techniques.

The cell of origin in the gizzard shad neoplasm has not been definitively determined [16,17]. Two major possibilities considered are pigment cells and peripheral nerve sheath cells. Pigment cells, particularly melanocytes, were likely because the tumors were usually darkly pigmented, whereas the swirling patterns of the tumors suggested an origin from peripheral nerve sheath cells although poorly differentiated pigment cell neoplasms can express similar patterns. Tumor location analysis show the lesions are not randomly distributed but that most (79.5%) occur on the dorsal surface. The area of highest occurrence was from the occiput to the dorsal fin origin. This anterior-dorsal section was the location for 244 of the 577 (42.3%) tumors scored and also correlates with a high concentration of nerve sheath cells that arise from the neural crest.

immunocytochemical studies are underway to determine the cell of origin (Geter et al., in prep.).

In summary, the cause of an epizootic of dermal neoplasia in gizzard shad (*Dorosoma cepedianum*) from lakes in south-central Oklahoma and north-central Texas is unresolved. The present study suggests that an etiology from trace elements or radiation cannot be supported. Furthermore, tumor location analysis suggests that direct exposure to sediment-related carcinogens is not a likely cause of the neoplasia. Future studies will investigate the geographic range of the disease, examine if ultra-violet light may play a role in the gizzard shad tumors, determine tumor prevalence in other species including the threadfin shad and examine additional tumor specimens to determine the cell of origin.

Acknowledgments---We thank Drs. Gordon Cook, Carla Goad, Roman Lanno, Charles Passo, and Ron Van Den Bussche for constructive comments on the manuscript and data analysis. We are also indebted to Ann Durham-Jacobs, David Goad, Ron Kuehn, Colleen Schehl, Dee McClanahan, Chris Ostrander, Troy Mayhew, and James Bryant for their expert technical assistance. We also thank Ron Parker, Lyn Weems, Dr. John E. Welch, and the personnel of the Chickasaw National Recreational Area in Sulphur, Oklahoma for their encouragement and logistical support. These studies were supported by grants from the Oklahoma State University Environmental Institute, Oklahoma Water Resources Research Institute, the Oklahoma Department of Environmental Quality, the U.S. Geological Survey, the National Park Service, and the National Institutes of Health (CA54950 and CA58818) to GKO.

REFERENCES

1. **Harshbarger, J.C.** and **J.B. Clark.** 1990. Epizootic of neoplasms in bony fish of North America. *Sci. Total Environ.* **94**:1-32.

2. **Black, J.J.** and **P.C. Baumann.** 1991. Carcinogens and cancers in freshwater fishes. *Environ. Health Perspect.* **90**:27-33.

3. **Harshbarger, J.C., P.M. Spero,** and **N.M. Wolcott.** 1993. Neoplasms in wild fish from the marine ecosystem emphasizing environmental interactions. In J.A. Couch and J.W. Fournie, eds., *Pathobiology of Marine and Estuarine Organisms.* CRC Press, Florida.

4. **Vogelbein, W.K., J.W. Fournie, P.A. Van Veld,** and **R.J. Huggett.** 1990. Hepatic neoplasms in the mummichog (*Fundulus heteroclitus*) from a creosote-contaminated site. *Cancer Res.* **50**:5978-5986.

5. **Baumann, P.C.** and **M. Whittle.** 1988. The status of selected organics in the Laurentian Great Lakes: an overview of DDT, PCB's, dioxins, furans and aromatic hydrocarbons. *Aquat. Toxicol.* **11**:241-258.

6. **Hayes, M.A., I.R. Smith, T.H. Rushmore, T.L. Crane, C. Thorn, T.E. Kocal,** and **H.W. Ferguson.** 1990. Pathogenesis of skin and liver neoplasms in white suckers from industrially polluted areas in Lake Ontario. *Sci. Total Environ.* **94**:105-123.

7. **Black, J.J.** 1983. Field and laboratory studies of environmental carcinogenesis in Niagara River fish. *J. Great Lakes Res.* **9**:326-334.

8. **Sonstegard, R.A.** 1977. Environmental carcinogenesis studies in fishes of the Great Lakes of North America. *Ann. NY Acad. Sci.* **298**:261-269.

9. **Baumann, P.C.** 1984. Cancer in wild freshwater fish populations with emphasis on the Great Lakes. *J. Great Lakes Res.* **10**:251-253.

10. **Down, N.E., R.E. Peter,** and **J.F. Leatherland.** 1988. Gonadotropin content of the pituitary gland of gonadal tumor-bearing common carp x goldfish hybrids from the Great Lakes, as assessed by bioassay and radioimmunoassay. *Gen. Comp. Endocrinol.* **69**:288-300.

11. **Black, J.J., E.D. Evans, J.C. Harshbarger,** and **R.F. Zeigel.** 1982. Epizootic neoplasms in fishes from a lake polluted by copper mining wastes. *J. Nat. Cancer Inst.* **69**:915-926.

12. **Sonstegard, R.A.** 1976. Studies of the etiology and epizootiology of lymphosarcoma in Esox (*Esox lucius* L. and *Esox masquinongy*). In W.E. Ribelin and G. Migaki, eds., *The Pathology of Fishes*. University of Wisconsin Press, Wisconsin, pp. 907-924.

13. **Eaton, W.D.** and **M.L. Kent.** 1992. A retrovirus in chinook salmon (*Oncorhynchus*

tshawytscha) with plasmacytoid leukemia and evidence for the etiology of the disease. *Cancer Res.* **52**:6496-6500.

14. **Bowser, P.R., M.J. Wolfe, J.L. Forney,** and **G.A. Wooster.** 1988. Seasonal prevalence of skin tumors from walleye (*Stizostedion vitreum*) from Oneida Lake, New York. *J. Wild. Dis.* **24**:292-298.

15. **Schmale, M.C., G.T. Hensley,** and **L.R. Udey.** 1986. Neurofibromatosis in the bicolor damselfish (*Pomacentrus partitus*) as a model of von Recklinghausen neurofibromatosis. *Ann. NY Acad. Sci.* **486**:386-402.

16. **Ostrander, G.K., W.E. Hawkins, R.L. Kuehn, A.D. Jacobs, K.D. Berlin,** and **J. Pigg.** 1995. Pigmented subcutaneous spindle cell tumors in native gizzard shad (*Dorosoma cepedianum*). *Carcinogenesis.* **16**:1529-1535.

17. **Jacobs, A.D.** and **G.K. Ostrander.** 1995. Further investigations of the etiology of subcutaneous neoplasms in native gizzard shad (*Dorosoma cepedianum*). *Environ. Toxicol. Chem.* **14**:1781-1787.

18. **Heller, S.R.** and **G.W.A. Milne, eds.** 1985. *EPA/NIH Mass Spectral Data Base*, 5 vols and Suppl. 1 and 2. National Standards Reference Data System. National Institute of Standards and Technology, US Department of Commerce, Washington, DC.

19. **Hawkins, W.E., D.G. Gregory, A.D. Jacobs,** and **G.K. Ostrander.** 1996. Poorly differentiated mesenchymal neoplasm in the dermis of a white bass. *J. Aquat. Anim. Health.* **8**:150-154.

20. **Childress, W.M.** 1991. Standard-weight curves derived from Texas length and weight data. *N. Amer. J. Fish. Manage.* **11**:480-482.

21. **Fishbein, L., A. Furst,** and **M.A. Mehlman, eds.** 1987. *Genotoxic and Carcinogenic Metals: Environmental and Occupational Occurrence and Exposure.* New Jersey: Princeton Scientific Publishing, 1987.

22. **Sanchez-Cabeza, J.A.** and **Ll. Pujol.** 1995. A rapid method for the simultaneous determination of gross alpha and beta activities in water samples using a low background liquid scintillation counter. Health Phys. **68**:674-682.

23. **Sambrook, J., E.F. Fritsch,** and **T. Maniatis.** 1989. *Molecular Cloning: A Laboratory Manual.* Cold Springs Harbor Laboratory, Cold Springs Harbor, NY.

24. **Matioli, S.R.,** and **R.A. deBrito.** 1995. Obtaining genetic markers by using double-stringency PCR with microsatellites and arbitrary primers. *BioTechniques.* **19**:752-758.

25. **Lynch, M.** and **B.G. Milligan.** 1994. Analysis of population genetic structure with RAPD markers. *Molec. Ecol.* **3**:91-99.

26. **Wetton, J.H., R.E. Carter, D.T. Parkin,** and **D. Walters.** 1987. Demographic study of a wild house sparrow population by DNA fingerprinting. *Nature (Lond).* **327**:147-149. pp. 157-176.

27. **U.S. Environmental Protection Agency.** 1986. Water pollution control: national primary drinking water regulations; radionuclides. Fed. Reg. 189, 34836-62, Washington, DC.

28. **U.S. Environmental Protection Agency.** 1995. Drinking water regulations and health advisories. Fed. Reg. title 40, part 141, Washington, DC.

29. **Houlk, R.S.** and **J.J. Thomas.** 1988. Inductively coupled plasma mass spectrometry. *Mass Spectrom. Rev.* 7:425.

30. **Vanhoe, H., C. Vandecasteele, J. Versieck,** and **R. Dames.** 1989. Determination of iron, copper, zinc, rubidium, molybdenum and cesium in human serum by inductively coupled plasma mass spectrometry. *Anal. Chem.* **61**:1851.

31. **Gercken, B.** and **R.M. Barnes.** 1991. Determination of lead and other trace element species in blood by size exclusion chromatography and inductively coupled plasma/mass spectrometry. *Anal. Chem.* **63**:283.

32. **U.S. Environmental Protection Agency.** 1977. Guidelines for the pollutional

classification of Great Lakes harbor sediments. Region 10, Washington, DC.

33. **Oklahoma Department of Water Quality.** 1992. Water Quality Guidelines. Beneficial Uses and Criteria, part 3, 785:45-5-10.

34. **Eisler, R.** 1981. *Trace Metal Concentrations in Marine Organisms*. Pergamon Press, New York, pp. 493-623.

35. **U.S. Environmental Protection Agency.** 1986. Effects of radiation on aquatic organisms and radiobiological methodologies for effects assessment. EPA 520/1-85-016. Office of Radiation Programs, Washington, DC.

36. **Branson, C.C., A.L. Burwell,** and **G.W. Chase.** 1955. Uranium in Oklahoma. OK Geol. Survey Mineral Rep 27, pps. 22.

37. **Hill, J.W.** 1957. Uranium-bearing carbonaceous nodules of south-western Oklahoma. OK Geol. Survey Mineral Rep 33, pps. 6.

38. **Finch, W.I.** 1967. Geology of epigenetic uranium deposits in sandstone in the United States. U.S. Geol. Survey Proff Paper 538, pps. 121.

39. **Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafaiski,** and **S.V. Tingey.** 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl.*

Acids Res. **18**:6531-6535.

Table 1. Dates, total gizzard shad caught and percent of tumor-bearing shad per catch for Lake of the Arbuckles, Lake Texoma and Lake Murray, Oklahoma, USA.

Lake of the Arbuckles

Date	Shad caught	Tumor-bearing	% with tumors
6-23/25-95*	142	27	19.0
10-2/3-95	10	2	20.0
11-11/12-95	15	2	13.0
11-24/26-95	129	17	13.2
5-5-96	77	9	11.7
8-11/12-96	85	7	8.2
Totals	**1448**	**272**	**18.8**

Lake Texoma

Date	Shad caught	Tumor-bearing	% with tumors
5-6/10-96	81	12	14.8
5-13/17-96	16	2	12.5
5-21&23-96	498	85	17.1
8-13-96	65	12	18.4
Totals	**660**	**111**	**16.8**

Lake Murray

Date	Shad caught	Tumor-bearing	% with tumors
5-22/23-96	20	4	20

* Fish sampled before 1995 were previously reported [16,17].

Table 2. Location analysis of tumors reported as frequency of tumors per section from gizzard shad from Lake of the Arbuckles (N=243; 1.72 tumors/shad), Lake Texoma (N=103; 1.5 tumors/shad) and total from both lakes.

Section	Arbuckle	Texoma	Total
anterior-dorsal	39.05	50.10	42.29
mid-dorsal	17.62	12.10	16.12
posterior-dorsal	19.52	25.50	21.14
anterior-ventral	7.14	3.20	6.07
mid-ventral	9.38	0.60	6.93
posterior-ventral	7.38	7.60	7.45

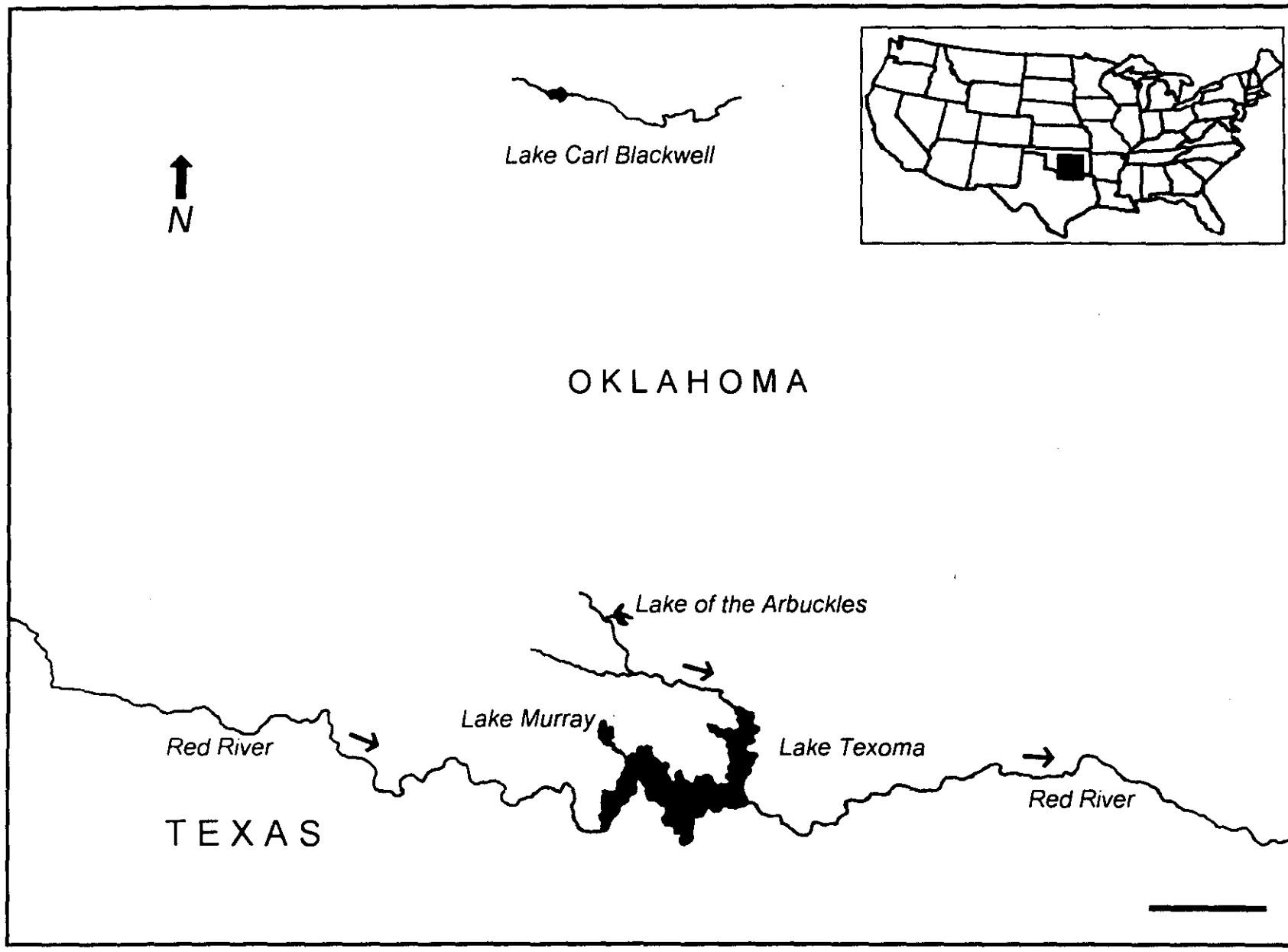
Lake Carl Blackwell
N
OKLAHOMA
Lake of the Arbuckles
Lake Murray
Red River
Lake Texoma
Red River
TEXAS

Figure 1. Map showing Lake of the Arbuckles, Lake Texoma, Lake Murray and the reference lake, Lake Carl Blackwell with arrows showing water flow. Bar equals 20 Km.