PIGMENTED SKIN TUMORS IN GIZZARD SHAD (DOROSOMA CEPEDIANUM) FROM THE SOUTH-CENTRAL UNITED STATES: RANGE EXTENSION AND FURTHER ETIOLOGICAL STUDIES

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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, USA. 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, Lake Murray and Lake Texoma in south-central Oklahoma, extending the range of the lesion. No neoplasms were found in shad from a reference site, Lake Carl Blackwell, Oklahoma. 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 levels. 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 2,128 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 of the epizootic.

Keywords—Fish Neoplasms Carcinogenesis Mass spectrometry Radiation

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 nonhepatic neoplasia is rarer than hepatic neoplasia and is not as clearly related to environmental contamination [1,3]. Epizootics of nonhepatic 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 nonhepatic as well as hepatic neoplasms in sauger (Sitzostedion canadense) and walleye (S. vitreum) [11]. Several epizootics of neoplasia appear to have a viral origin. These include lymphoma in northern pike (Esox lucius) [12], plasmacytoid leukemia in chinook salmon (Oncorhynchus tschawytscha) [13], dermal sarcoma in walleye [14], and neurofibromatosis in bicolor dace (Pomacentrus partitus) [15].

The present study involved an epizootic of pigmented subcutaneous spindle-cell neoplasms previously reported in gizzard shad (Dorosoma cepedianum) from Lake of the Arbuckles, a manmade lake in central Oklahoma, USA [16,17]. In those studies, lesions were found in about 22% of adult gizzard shad and in 0% of nearly 2,000 juvenile shad examined. The occurrence of lesions did not appear to be seasonal. Likewise, there were no differences in tumor incidence between adult males and females. Results of transmissibility studies conducted with rainbow trout injected with cell-free extracts of gizzard shad neoplasm were negative [17]. Ostrander et al. [16] described the appearance, distribution, and histological characteristics 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.
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Fractionation of lake water and sediment samples followed by gas chromatography–mass spectrometry analysis revealed no carcinogenic compounds when compared to the spectra in the National Institute of Standards and Technology (NIST) database [18], in which more than 42,000 compounds are archived. Results of 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 hemangio-pericytoma from a white bass (Morone chrysops) [19], and poorly differentiated spindle-cell neoplasms from two threadfin shad (D. petenense) (D.R. Geter et al., unpublished data).

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) whether 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) whether 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 origin.

MATERIALS AND METHODS

Materials

Unless noted otherwise, analytical-grade reagents (Sigma Chemical, 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 (Fig. 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 (310–490 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 h for 24 to 72 h. Netted gizzard shad were weighed, measured, and examined grossly for tumors. Liver and muscle tissue were excised for ICP–MS analysis, and excised liver tissue was also analyzed by RAPD and DS–PCR.

Inductively coupled plasma mass spectrometry

The 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 were obtained with a Van Dorn sampler, and epilimnetic water samples were obtained by hand 5 cm below the water surface. Sediment and water samples were placed in 50-ml acid-washed polyethylene tubes (Fisher Scientific, Pittsburgh, PA, USA). Samples and blanks were placed on ice, transported to the laboratory in darkness, and stored at 4°C for 2 d in darkness before shipping. Water samples and trip blanks were filtered with Whatman glass microfiber filters. Filtered water and filter were placed in separate precleaned, acid-washed, 100-ml glass sample vials wrapped in aluminum. About 1.0 to 1.5 g (wet weight) of both liver and muscle was 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, 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 used in these procedures were of ultrapure grade to minimize the introduction of metals. The NIST standard reference materials 1646 (estuarine sediment) and 1635 (oyster tissue) were analyzed in parallel with each set of samples to control for contamination and to 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 a U.S. Environmental Protection Agency (EPA) 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 after a 1/10 dilution in 3% ultrapure nitric acid solution, and samples were analyzed within 1 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 microwave digestion system (CEM Corporation, Matthews, NC, USA). Digestions were performed using approx. 1 g (wet weight) of sediment or tissue in 10 ml of 50% ultrapure nitric acid for approx. 2 hours. Samples were diluted to a final volume of 50 ml, and a 1-ml aliquot was analyzed for trace element concentrations. Samples of Milli-Q® water (Milli-Q, Bedford, MA, USA) 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 mass spectrometer. Samples were analyzed using triplicate, 1-min data acquisition/integration times. Final trace element concentrations were blank subtracted and corrected for internal standard recovery, analysis dilution, digestion volume, and original mass of the sample.

Environmental radiation

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

RAPD and DS–PCR analyses

Tumor-bearing and nontumor-bearing gizzard shad from Lake of the Arbuckles were weighed and measured, and their livers were excised. Dissecting scissors were soaked in 100% ethanol and thoroughly cleaned before each dissection. Tissues were individually wrapped in 30- × 30-cm sheets of autoclaved aluminum foil, placed in plastic freezer bags, kept on dry ice until they were brought to the laboratory, and stored at −20°C. Extraction of DNA was accomplished by standard phenol/chloroform separation, followed by ethanol precipitation; DNA was stored in a tris (hydroxymethyl) aminomethane (Tris)—ethylenediaminetetra-acetic acid (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 (CTCCACCCRCRCRAGT) core microsatellite primer (Oklahoma State University Recombinant DNA/Protein Resource Facility, Stillwater, OK, USA) amplifies a region between microsatellites, whereas the second primer, a standard 10-mer RAPD primer (Operon Technologies, Alameda, CA, USA) 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× TBE (9 mM Tris-borate and 0.2 mM EDTA, pH 8.0) at 25 V for 5 h in 5.0% polyacrylamide [23], stained with ethidium bromide, examined under ultraviolet 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 1 μg of a 100-bp size standard (15628–050, Gibco-BRL, Gaithersburg, MD, USA). 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 = 2N/aN/a + N/a, where N/a is the number of shared bands, N/a is the number of bands in one lane, and N/a 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), and tumor-bearing versus nontumor-bearing shad (lanes 1–4 vs 5–8), and BSs of all individuals were compared (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 anatomic 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 had 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 seven years. Extensive analyses of all tumors were conducted initially. After looking at more than 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; the shad 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 posterodorsal section, and all ventral fins were included in the posteroverentral section to assess tumor occurrence in fins.

Statistics

Fisher’s exact test was used to determine differences in neoplasm occurrence in the gizzard shad populations between
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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 results from previous studies and those from 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 Lebanon (n = 498, 17.1%) sites. Gizzard shad (n = 44 adults and 200 juveniles, all studies) collected from Lake Carl Blackwell did not exhibit grossly observable neoplasms.

Inductively coupled plasma mass spectroscopy

The 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 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 liver and nickel (10.35 vs 4.48 μg/g) in muscle.

Environmental radiation

Radioactivity levels in 45 water samples from Lake of the Arbuckles, Lake Texoma, and Lake Carl Blackwell ranged from less than 0.07 to 0.51 Bq/L for alpha, from less than 0.40 to 1.60 Bq/L for beta, and <100 pCi/L for radon-222 radiation. All samples were well below EPA guidelines for alpha, beta, and radon radiation [27, 28].

RAPD and DS–PCR analyses

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 in the ventral section (118/577, 20.5%) (p = 0.001). Lesions were particularly abundant in the anterior-dorsal portion of the fish with an occurrence of 42.3% (244/577, 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

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

<table>
<thead>
<tr>
<th>Date</th>
<th>Shad caught</th>
<th>Tumor-bearing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake of the Arbuckles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/23–25/95*</td>
<td>142</td>
<td>27</td>
</tr>
<tr>
<td>10/2–3/95</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>11/11–12/95</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>11/24–26/95</td>
<td>129</td>
<td>17</td>
</tr>
<tr>
<td>5/5/96</td>
<td>77</td>
<td>9</td>
</tr>
<tr>
<td>8/11–12/96</td>
<td>85</td>
<td>7</td>
</tr>
<tr>
<td>Totals</td>
<td>1,448</td>
<td>272</td>
</tr>
<tr>
<td>Lake Texoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5/6–10/96</td>
<td>81</td>
<td>12</td>
</tr>
<tr>
<td>5/13–17/96</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>5/21, 23/96</td>
<td>498</td>
<td>85</td>
</tr>
<tr>
<td>8/13/96</td>
<td>65</td>
<td>12</td>
</tr>
<tr>
<td>Totals</td>
<td>660</td>
<td>111</td>
</tr>
<tr>
<td>Lake Murray</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5/22–23/96</td>
<td>20</td>
<td>4</td>
</tr>
</tbody>
</table>

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

Table 2. Location analysis of tumors in gizzard shad from Lake of the Arbuckles, Lake Texoma, and both lakesa

<table>
<thead>
<tr>
<th>Section</th>
<th>Lake of the Arbuckles</th>
<th>Lake Texoma</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior-dorsal</td>
<td>39.05</td>
<td>50.10</td>
<td>42.29</td>
</tr>
<tr>
<td>Middorsal</td>
<td>17.62</td>
<td>12.10</td>
<td>16.12</td>
</tr>
<tr>
<td>Posterior-dorsal</td>
<td>19.52</td>
<td>25.50</td>
<td>21.14</td>
</tr>
<tr>
<td>Anterior-ventral</td>
<td>7.14</td>
<td>3.20</td>
<td>6.07</td>
</tr>
<tr>
<td>Midventral</td>
<td>9.38</td>
<td>0.60</td>
<td>6.93</td>
</tr>
<tr>
<td>Posterior-ventral</td>
<td>7.38</td>
<td>7.60</td>
<td>7.45</td>
</tr>
</tbody>
</table>

a Reported as frequency of tumors per section. For Lake of the Arbuckles, n = 243 and 1.72 tumors/shad were found. For Lake Texoma, n = 103 and 1.5 tumors/shad were found.
sections. About one-third of the tumor-bearing gizzard shad had multiple tumors (117/346). 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 (*D. 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, Lake Texoma and Lake Murray, which are about 55 km south of Lake of the Arbuckles but share drainages. The reference site, Lake Carl Blackwell, is 180 km north, lies in a different drainage, and remains free of tumor-bearing fish. The purpose of this article is not to define with the highest level of confidence the tumor incidence in these additional lakes. Instead, it is to report that unusual tumors are found at other locations in the drainage basin. Neither the geographic extent nor the date when 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, 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]. The ICP–MS analyses of the sediment and water samples revealed trace element concentrations below levels suggested by both the 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 levels in shad to be within average concentrations [34]. Although we cannot account for the differences in concentrations of beryllium and nickel in tumor-bearing versus nontumor-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 contributor 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 to 70 ppm uranium. Also, local deposits of crude oil and asphalt contained higher than normal amounts of uranium [36–38]. 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 EPA drinking-water guidelines [27,28], suggesting that radiation probably is not a factor in the gizzard shad neoplasms.

Random amplified polymorphic DNA and DS–PCR were used to generate a genetic marker to identify and separate tumor-bearing and nontumor-bearing gizzard shad. Random amplified polymorphic DNA 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 analyses, a genetic marker was not identified, and no statistical difference was observed 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 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 showed that 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 origin of the dorsal fin. 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 arising from the neural crest. However, pigment cells also arise from the neural crest, and the dorsal area of the gizzard shad is more heavily pigmented than the ventral area. Further histopathological and immunocytochemical studies are under way to determine the cell of origin (D.R. Geter et al., unpublished data).

In summary, the cause of an epizootic of dermal neoplasia in gizzard shad (*D. cepedianum*) from lakes in south-central Oklahoma and north-central Texas is unresolved. Results of the present study suggest that an etiology from trace elements or radiation cannot be supported. Furthermore, results of tumor location analysis suggest 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 whether ultraviolet light plays 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.

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