



Figure 1. An adult gizzard shad exhibiting multiple raised black tumors.

Persistent expression of tumors in Lake of the Arbuckles gizzard shad

A summary of eight years of study

By GARY K. OSTRANDER, RON PARKER, AND WILLIAM E. HAWKINS

During the spring of 1991, while conducting studies on the health of fishes in various lakes and rivers in Oklahoma (e.g., Kuehn et al. 1995), we were asked to investigate reports of large black tumors appearing on the skin of gizzard shad (*Dorosoma cepedianum*—fig. 1). The shad were collected from a lake within Chickasaw National Recreation Area in south-central Oklahoma. The Lake of the Arbuckles is a 2,350-acre reservoir fed by Guy Sandy Creek, Buckhorn Creek, Rock Creek, and its tributary, Travertine Creek (fig. 2). The lake has a mean depth of 9 m (~30 ft) and 58 km (36 mi) of shoreline. In 1996, the park recorded more than 70,000+ boater visits and approximately 20,000 boats on the lake with most visitors participating in sport fishing activities. The primary sport fish within the recreation area include largemouth, smallmouth, and spotted bass; crappie; channel catfish; and sunfish. The incidence of tumors on gizzard shad is of concern because the species is a forage fish in Lake of the Arbuckles, the park's largest aquatic resource, and it is unexplained.

Construction and filling of the Lake of the Arbuckles was completed in 1967 and nothing in its history suggests any significant contamination could be responsible for tumors appearing in the fish. Nonetheless, we began a comprehensive study in August of 1991 with the ongoing objective of determining the nature and extent of the lesions appearing in the gizzard shad residing in the lake.

Malignant tumors documented

The initial survey of Lake of the Arbuckles revealed that 14 of 105 gizzard shad collected exhibited one or more raised black lesions. Grossly, the lesions were primarily distributed over the head, trunk, and fins as superficial raised masses that were almost always darkly pigmented (see fig. 1) and ranged in size from 0.1 to >2.0 cm (0.04–0.79 in) in diameter. The lesions were ultimately diagnosed as subcutaneous spindle cell tumors that most likely arose from the cells that surround nerves or from pigment cells (Ostrander et al. 1995).

Subsequent surveys of the lake (1991–96) and the collection of over 1,200 adult and at least 2,000 juvenile shad have revealed that at any one time approximately 20% of the adult shad (>1 year) exhibit one or more tumors. To date, tumors have not been seen in any of the juvenile shad that we have examined. The significance of these observations lies in the fact that fish exhibiting tumors in 1996 were not present when the lesions were first discovered in 1991; thus, whatever is responsible for the formation of the tumors is still exerting its effect today.

What is causing the tumors?

Once the diagnosis of malignant cancerous tumors in the gizzard shad was made, the focus of our efforts shifted to attempting to determine the cause. Cancer in fishes has been previously reported at various locations in North America (e.g., Puget Sound, Boston Harbor, The Great Lakes), but never in a national park (reviewed in

Harshbarger and Clark 1990). Typically, tumor-bearing fish populations are found in areas high in human-caused pollution with aromatic hydrocarbons (e.g., fossil fuels) or heavy metals most often being implicated; thus, our initial studies focused on the analysis of the water and sediments of the Lake of the Arbuckles. Water and sediment samples were collected from a variety of locations and subjected to gas chromatography-mass spectrometry. The resulting spectra were matched to an online library of 44,000 environmental contaminants. No significant matches were found. Likewise, analysis of water, sediment, and shad tissue samples did not reveal significant levels of chromium, nickel, lead, cadmium, or copper—all previously implicated in tumor formation in other vertebrates, including humans. Finally, we used inductively coupled plasma mass spectrometry to determine if significant levels of 64 trace elements (e.g., arsenic, selenium, mercury, etc.) correlated with the high levels of tumors seen in the gizzard shad. Again, no significant increase in any of these elements was observed (Ostrander et al. 1995, Jacobs and Ostrander 1995).

The region around the Lake of the Arbuckles is the site of natural deposits of uranium and as such we explored the hypothesis that elevated levels of radioactive uranium or its by-product radon gas could be contributing to the high tumor incidence seen in the Lake of the Arbuckles. Water samples were collected from Lake of the Arbuckles and Lake Carl Blackwell and analyzed for gross alpha/beta and radon-222 radiation. Lake Carl Blackwell is also located in central Oklahoma and contains a large population of tumor-free shad. As with the other chemical analyses, no increase in the background levels of uranium, total radioactivity, or radon were observed. Moreover, no differences were noted between the two lakes (Geter et al. 1998).



Figure 2. Map of south-central Oklahoma and northern Texas illustrating sampling sites. Lake of the Arbuckles is within Chickasaw National Recreation Area.

According to earlier research, viruses can cause tumors in fish and other organisms. Among fishes, at least two examples of tumors similar to what we are observing in the Lake of the Arbuckles have been linked to retroviruses (discussed in Ostrander et al. 1995). Our laboratory developed a test for the assessment of a retroviral cause of these tumors. Examination of the tumors for reverse transcriptase, an enzyme indicative of the presence of a retrovirus, was negative. Likewise, analysis of the tumors by electron microscopy failed to reveal the presence of any retroviral or other viral particles. Along these same lines, the occurrence of tumors was not seasonal as often is the case with virally induced cancers in feral fish population (Ostrander et al. 1995).

We have also investigated the possibility that tumors in the gizzard shad may arise from only a certain segment of the population. That is, it is reasonable to hypothesize that if restricted interbreeding was occurring within a segment of the population the tumor phenotype could be carried through successive generations. The mechanism for such an occurrence has been previously identified in fish and humans. The best studied example is the occurrence of melanomas in laboratory populations of *Xiphophorus* (swordtails and platys—fish that bear live offspring) that are caused by the altered expression of a tumor suppressor gene (reviewed in Ostrander and Blair 1997). Altered expression of tumor suppres-

sor genes and oncogenes have been implicated in a variety of human cancers including those of the breast, ovary, kidney, and eye. We have recently completed pilot studies in which we examined tumor-bearing and nontumor-bearing individuals for obvious genetic markers. Specifically, we performed random amplified polymorphic DNA (RAPD) and double-stringency polymerase chain reaction (DS PCR—laboratory techniques that facilitate detection of DNA-based diseases) analysis (described in Geter et al. 1998). Tumor-bearing gizzard shad were indistinguishable from nontumor-bearing gizzard shad by genetic marker analysis performed in our studies.

Cancer in fishes has been previously reported at various locations in North America, but never in a national park

Field studies, in which fish were sampled from various locations at different times of the year supported the hypothesis that tumor-bearing and nontumor-bearing shad collected from Lake of the Arbuckles represent a single genetically homogeneous population (Jacobs and Ostrander 1995).

Tumors in other fish?

The primary focus of our studies for the last six years has been the gizzard shad and, as such, our sampling methods are optimized to target this species. Nonetheless, we often capture nontarget species in our

nets. These fish are routinely examined grossly for tumors and on occasion complete necropsy is performed. The most common non-shad species caught is the catfish, and to date no tumors have been seen. Over the years we have also caught about 30 bass, including white, smallmouth, and largemouth. Two individuals have presented tumors and one of these has been examined in some detail (Hawkins et al. 1996). A white bass exhibited a tumor that was a solitary soft round mass that bulged from the anal fin. The lesion was suggestive of a poorly differentiated hemangiopericytoma (a tumor that likely arose from cells surrounding a blood vessel), though it might have derived from a nerve sheath, pigment cells, fibroblasts, or smooth muscle. Hemangiopericytoma is a relatively rare lesion in wild fish. Its discovery in a white bass from the same location in which resident gizzard shad exhibit a high frequency of similar lesions arising from cells around nerves instead of blood vessels is of concern. Further surveys and studies of non-shad species are needed.

Unlike hemangiopericytoma, pigmented subcutaneous spindle cell neoplasm is a tumor that arises from cells surrounding nerves. Tumors of this type were first observed in gizzard shad in 1991 and thought to be limited to Lake of the Arbuckles; however, a similar incidence (~20%) of this disease has now been documented in three additional lakes. Two of these lakes, Texoma and Murray, are located about 55 km (34 mi) south of Lake of the Arbuckles and share the same drainage. Sampling was conducted at multiple sites at lake Texoma (Glasses, Caney, and Lebanon) and one site

at Lake Murray (see fig. 2). Both Lake of the Arbuckles and Lake Murray were stocked by the Oklahoma Department of Water Quality with gizzard shad from Lake Texoma in 1980 (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; that is, they may have a common ancestor that carried this deleterious trait. A third lake, Fort Supply, is found in the

See "Shad" on page 36

western panhandle region and is outside this drainage. In all cases the incidence of tumors remains roughly 20%.

hemangiopericytoma in a white bass, *Monroa chrysops*. Journal of Aquatic Animal Health. 8:150-54.

Ostrander, G. K., W. E. Hawkins, R. L. Kuehn, S. D. Jacobs, K. D. Berlin, and J. Pigg. 1995. Epizootic



For the study, researchers collected juvenile and adult shad from Lake of the Arbuckles with a beach seine.

Future direction

To date we have not been able to determine the cause or source of the tumors appearing in gizzard shad in four Oklahoma lakes. Moreover, neither the geographical extent of the outbreak nor when it began are known. Finally, the exact cell(s) of origin for the tumors remains to be determined. Our current studies are focused on answering these questions. **P**

Literature Cited

Geter, D. R., W. E. Hawkins, J. C. Means, and G. K. Ostrander. 1998. Pigmented subcutaneous spindle cell tumors in gizzard shad (*Dorosoma cepedianum*): Occurrence in two additional lakes and further investigations into the etiology of the lesions. Environmental Toxicology and Chemistry 17(11):2282-87.

Jacobs, A. D. and G. K. Ostrander. 1995. Etiological studies of subcutaneous neoplasms in feral gizzard shad, *Dorosoma cepedianum*. Environmental Toxicology and Chemistry 14:1781-87.

Kuehn, R. L., K. D. Berlin, W. E. Hawkins, and G. K. Ostrander. 1995. Anthropogenic contaminants and fish health near three oil refineries. Journal of Toxicology and Environmental Health 46:101-16.

Harshbarger, J. C. and J. B. Clark. 1990. Epizootiology of neoplasms in bony fish of North America. Science of the Total Environment 94:1-32.

Hawkins, W. E., D. G. Gregory, A. D. Jacobs, and G. K. Ostrander. 1996. Unusual dermal

of peripheral nerve sheath tumors in feral gizzard shad (*Dorosoma cepedianum*). Carcinogenesis 16:1529-35.

Ostrander, G. K., and J. B. Blair. 1997. The role of growth factors during teleost carcinogenesis. Pages 73-78 in L. Rossi, R. Richardson, and J. Harshbarger, editors. Spontaneous animal tumors: A survey.

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