FURTHER STUDIES OF PIGMENTED SUBCUTANEOUS SPINDLE CELL NEOPLASMS IN GIZZARD SHAD, *DOROSOMA CEPEDIANUM*, RESIDING IN LAKE OF THE ARBUCKLES, MURRAY COUNTY, OKLAHOMA

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IN GIZZARD SHAD, *DOROSOMA CEPEDIANUM*,

RESIDING IN LAKE OF THE ARBUCKLES,

MURRAY COUNTY, OKLAHOMA

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PREFACE

The objectives of this study were to (1) survey the incidence of peripheral subcutaneous spindle cell neoplasms in gizzard shad, *Dorosoma cepedianum*, residing in Lake of the Arbuckles, Murray county, Oklahoma; (2) assess possible retroviral etiology of this disease by assaying malignant tissues for reverse transcriptase activity; (3) measure the concentrations of cadmium, chromium, copper, nickel, and lead in water, sediment, and gizzard shad livers by graphite furnace atomic absorption; and (4) determine transmissibility of gizzard shad peripheral subcutaneous spindle cell neoplasms into healthy rainbow trout, *Oncorhynchus mykiss*. This thesis has been prepared as two chapters that have been submitted as two manuscripts. The first manuscript was submitted with collaborators to *Cancer Research* and the second manuscript was submitted to *Environmental Toxicology and Chemistry*.

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ABBREVIATIONS

Chapter II Terms

PCB's	polychlorinated biphenyls
RT	reverse transcriptase
PL	plasmacytoid leukemia
DNF	damselfish neurofibroma
GSN	gizzard shad neoplasm(s)
BTEX	benzene, toluene, ethylbenzene, and xylenes
TNES	10 m*M* Tris-HCl, pH 7.5, 100 m*M* NaCl, 1.0 m*M* EDTA [ethylenediamine tetracetic acid], 5% sucrose
TNE	10 m*M* Tris-HCl, pH 7.5, 100 m*M* NaCl, 1.0 m*M* EDTA
DTT	dithiothreitol
TCA	trichloroacetic acid
MMLV-RT	Moloney murine leukemia virus reverse transcriptase.
CPM	counts-per-minute

Chapter III Terms

GSN	gizzard shad neoplasm(s)
CHV	Cyprinid herpesvirus-1
GFAA	graphite furnace atomic absorption
EPA	Environmental Protection Agency

PBS	phosphate buffered saline
ANOVA	analysis of variance
LSD	least significant difference
LHV	Lucké herpesvirus
BDL	below detection limits

CHAPTER I

INTRODUCTION

The specific aims of this study were to (1) survey the incidence of peripheral subcutaneous spindle cell neoplasms (referred to as gizzard shad neoplasms, GSN) in, *Dorosoma cepedianum*, residing in Lake of the Arbuckles, Oklahoma; (2) assess possible retroviral etiology of this disease by assaying malignant tissues for reverse transcriptase activity; (3) measure the concentrations of cadmium, chromium, copper, nickel, and lead in water, sediment, and gizzard shad livers from Lake of the Arbuckles and Lake Carl Blackwell by graphite furnace atomic absorption; and (4) determine transmissibility of gizzard shad peripheral subcutaneous spindle cell neoplasms into healthy rainbow trout, *Oncorhynchus mykiss*. This thesis has been prepared as two chapters that have been submitted as two manuscripts. The Chapter II was submitted with collaborators to *Cancer Research* and the Chapter III was submitted to *Environmental Toxicology and Chemistry*. Differences in the format of Chapters II and III are due to specific journal style preferences. Certain passages of Chapter II, as well as Table 1 and Figure 1 were repeated in Chapter III for continuity.

CHAPTER II

PIGMENTED SUBCUTANEOUS SPINDLE CELL NEOPLASMS IN GIZZARD SHAD, *DOROSOMA CEPEDIANUM*

ABSTRACT

Greater than 22% of the adult gizzard shad (*Dorosoma cepedianum*), collected from Lake of the Arbuckles in south central Oklahoma over a two and one-half year period, exhibited raised, darkened lesions. Following complete histopathological examination, lesions were diagnosed as pigmented subcutaneous spindle cell neoplasms. This is the first report of an epizootic of pigmented subcutaneous spindle cell neoplasms occurring in a freshwater fish. Retroviruses have been associated with neoplastic lesions in some wild fish populations. Homogenates of neoplastic tissues were evaluated for retroviral infection by an assay for reverse transcriptase, and examined by electron microscopy. No evidence of retroviral infection was found.

INTRODUCTION

The occurrence of epizootic neoplasms among teleost fishes has been widely documented throughout North America (1). Over forty locations in North America with significant concentrations of anthropogenic pollutants have been linked to high occurrences of feral fish neoplasms (1). In 1975, McCain *et al.* found that 32% (20 out of 62) of the English sole (*Parophrys vetulus*) collected from Duwamish River Estuary in Seattle, Washington, exhibited liver lesions (2). Upon closer examination, Pierce *et al.* (3) noted that 92% (57 out of 62) of the English sole from the Duwamish River Estuary exhibited liver abnormalities such as fatty vacuolation, congestion, structure disarray, necrosis, and hepatomas. Due to these findings, Malins *et al.* (4) began one of the most comprehensive multidisciplinary studies of the relationships between anthropogenic contaminants and fish diseases found in the Puget Sound, WA, U.S.A. They discovered that fish collected in urbanized bays had consistently higher prevalences of liver lesions as compared to fish collected in nonurbanized bays (4). Detailed analyses of urban site sediments revealed more than 900 organic compounds. Urbanized sites also had at least 150 times higher mean concentrations of aromatic hydrocarbons than nonurban sites (4). English sole with hepatic neoplasms were collected at sites with high sediment concentrations of aromatic hydrocarbons. As further evidence of hydrocarbon exposure, metabolites of hydrocarbons resistant to biotransformation were detected in

the bile of English sole collected in urban waterways (5). However, the correlation between sediment concentration of toxic chemicals and the incidence of neoplasms cannot be interpreted as a conclusive cause-and-effect relationship.

Studies of feral fish neoplasms at other sites have revealed similar effects to what has been reported in the Puget Sound. Several sites within San Diego Bay were determined to be among the most polluted sites on the West Coast (6). That designation was due to high concentrations of aromatic hydrocarbons, polychlorinated biphenyls (PCB's), copper, and lead in the sediments. Likewise substantial concentrations of PCB's were reported in fish livers as well as a high prevalence of fin erosion (6). The white croaker (*Genyonemus lineatus*) (3.5%, 6 out of 172) and the black croaker (*Cheilotrema saturnum*) (10.2%, 9 out of 88) collected within the bay exhibited liver lesions such as foci of cellular alteration and hepatocellular nuclear pleomorphism. Species collected outside the bay did not possess liver lesions.

Many sites along the Atlantic coast of North America are also inundated with anthropogenic contaminants. Such sites include Chesapeake Bay, New Bedford Harbor, Long Island Sound, Massachusetts Bay, Narragansett Bay, New York Bight, Hudson River-New York Harbor complex, and tributaries of the Connecticut River (7). These areas are highly populated centers for industry and manufacturing (8). Aromatic

hydrocarbons, PCB's, pesticides, heavy metals, and phthalate esters were commonly present in sediments and fish tissues collected from New England, Long Island Sound, the Hudson River Estuary/New York Bight and the Elizabeth River (7). For example, Atlantic tomcod, *Microgadus tomcod*, in the Hudson river system were collected in five age classes. However, in 1979, the older age classes (3+ and 4+ years) were not as abundant as were previously reported (9). Smith and *et al.* (9) suggested that the population alteration may have been due to the duration of exposure to anthropogenic pollutants which increased the mortality of the older age classes. Over 85% of the tomcod in age classes 2+, 3+, and 4+ collected in the Hudson River system exhibited hepatocellular carcinomas (9).

Comparatively few extensive studies have been conducted concerning cancer in freshwater fish species. Liver lesions have been described in the brown bullhead (*Ictalurus nebulosus*) collected from the Black River, Ohio (10). Over 38% (48 out of 125) of the bullheads collected in 1982 exhibited cholangiocarcinomas and hepatocarcinomas. Preneoplastic liver changes were present in 84% of all fish collected in 1982. The sediments analyzed from the Black River had 1,000 times higher polynuclear aromatic hydrocarbon levels than those sediments collected from the reference site (10).

The Great Lakes region was well known for commercial fishing and clean water, but in the last two decades, anthropogenic pollutants have led

to the deterioration in water quality and an increase in the reports of neoplasia in feral fish populations (11). According to the Registry of Tumors in Lower Animals, 16 of the 41 epizootics reported in North America came from the Great Lakes vicinity, and 12 species were commonly affected (1). For instance, the two-year-old brown bullheads, *Ictalurus nebulosus*, collected from a Lake Erie tributary had a tumor rate of 1.2%, whereas those bullheads three years and older had a tumor rate of 33% and exhibited hepatic cholangiocarcinomas, cholangiomas, and adenofibromas (12). As early as 1977, Sonstegard, concluded that the high incidence of neoplasms were the result of increasing industrialization because 25% of all manufacturing in North America occurred in that region (13).

The previously described cases of feral fish with cancer have two similarities, namely that most epizootics were hepatocellular and each site had detectable levels of anthropogenic pollutants (1-13). However, much less is known about non-liver epizootics of cancer in feral fish. Furthermore, some epizootics of cancer do not appear linked to anthropogenic contaminants. Three such cases are the lymphosarcoma in the northern pike (*Esox lucius*), the dermal sarcoma of the walleye (*Stizostedion vitreum*), and plasmacytoid leukemia in chinook salmon (*Oncorhynchus tschawytscha*) (14-16).

Lymphosarcomas were seasonally present in 20.9% of the adult northern pike collected throughout North America (14). According to

Sonstegard, (17) lymphosarcomas were transmitted during spawning, and hence its high prevalence in early spring. Mulcahy and O'Leary (18) were successful in their attempts to transmit the disease into healthy northern pike. A cell-free extract of lymphosarcoma tissue was injected into healthy northern pike and, within 48 to 55 days, injected pike showed histological changes consistent with spontaneous lymphosarcoma (18). In further attempts to understand the etiology of this disease, Papas and Sonstegard (14) homogenized malignant tissues and subjected them to sucrose gradient centrifugation and fractionation. Aliquots of the fractions were assayed for reverse transcriptase (RT) activity as evidence of retroviral infection. Reverse transcriptase activity was associated with the fraction with a density of 1.16 g/ml. This was the first report of reverse transcriptase (i.e. a retrovirus) in a fish (14).

Approximately 27% of the adult walleye collected throughout North America are seasonally afflicted with benign dermal sarcomas (15). Dermal sarcomas were most prevalent during early spring and late autumn. Martineau *et al.* (1990) conducted experimental transmission experiments with fingerling walleye (19). Cell-free filtrates of sonicated tumor cells induced dermal sarcomas in walleye four months post-injection, further strengthening a viral etiology hypothesis (19). Malignant dermal tissue was homogenized and subjected to sucrose gradient centrifugation, serial fractionation, and was then assayed for RT activity (20). Reverse

transcriptase activity was detected in fractions with a density of 1.18 g/ml, which was consistent with other C-type retroviruses (14).

Plasmacytoid leukemia (PL) has been responsible for considerable mortalities of chinook salmon reared in seawater netpen facilities located in Western British Columbia, Canada, since 1988 (16). Tissues of the eye and kidney of pen-reared chinook salmon exhibiting PL were isolated and analyzed for RT activity (16). Samples with buoyant densities of 1.16 to 1.18 g/ml showed RT activity. These findings were consistent with other reports of retroviral infection in other fish species (14, 20, 21). Tissues from the liver, kidney, spleen, gut and/or mesenteries of individuals with PL were homogenized and passed through a 0.22 μm filter to remove whole cells and bacteria. Injections of PL cell-free filtrates induced PL in 70% of the healthy fish inoculated (22).

Preliminary reports of nerve sheath lesions of the bicolor damselfish (*Pomacentrus partitus*) are another case of feral fish with cancer that does not appear associated with anthropogenic pollutants (23). Schmale *et al.* (23) characterized the disease and termed it damselfish neurofibroma (DNF). Neoplasm incidences ranged from 0.4 to 23% of the sexually mature adults collected. Schmale and Hensley (24) conducted transmissibility studies and found that 84% of the healthy individuals injected with cell-free neoplasm extracts developed neurofibroma lesions. Lesions appeared within 5 months of inoculation for juveniles and 14 months for adults. Further examination

revealed that induced neoplasms were caused by the neoplastic conversion of host nerve cells rather than the growth of introduced neoplastic tissue. Schmale and Hensley (24) also suggested that transmission of DNF may be the result of retroviral infection. According to Schmale, preliminary results indicated that RT activity was present in cell cultures of neurofibroma tissue (25).

This report is the first account of a pigmented subcutaneous spindle cell neoplasm (referred to as gizzard shad neoplasm, GSN) in a fresh water species, the gizzard shad (*Dorosoma cepedianum*) (26, 27). Adult gizzard shad collected from Lake of the Arbuckles, Murray county, Oklahoma, between August, 1991, to September, 1993, exhibited raised, darkened lesions. Previous analysis of water and sediments by gas chromatography/mass spectrometry did not reveal any carcinogenic aromatic hydrocarbons (26, 27). BTEX analysis (benzene, toluene, ethylbenzene and xylene) did not detect concentrations of benzene, toluene, ethylbenzene, or xylene above 5 ppm (26, 27).

In response to preliminary studies, further experiments were conducted. More field collections were made to determine the epidemiology of this disease. A reverse transcriptase assay was performed on homogenates of malignant tissues to evaluate potential retroviral infection.

MATERIALS AND METHODS

Study Site. Lake of the Arbuckles is a man-made lake located in Murray county of south central Oklahoma (Fig. 1). It was completed in 1967 to serve as a recreational lake and a source of water for the two neighboring towns, of Sulfur and Davis, with populations of 5,500 and 2,800, respectively. It is formed at the confluence of the Guy Sandy, Rock, and Buckhorn creeks, is encompassed by 58 km of shoreline, and has a mean depth of 9.4 m.

Upon completion in 1938, Lake Carl Blackwell, a man-made impoundment located 14 km west of Stillwater in north central Oklahoma, served as a flood control system and as a recreational lake for Payne county (Fig. 1). Stillwater Creek was dammed as part of the Federal Government Land Utilization Project. Lake Carl Blackwell has a mean depth of 4.93 m and is encompassed by 88.5 km of shoreline.

Epidemiology. Adult gizzard shad were collected at 1 to 4 month intervals from Lake of the Arbuckles, Murray county, Oklahoma, with 91 m gill nets with 10 cm stretched-mesh. The nets were set at depths ranging from 1.8 to 4.3 m at three different sites. Nets were checked approximately every two hours during daylight hours and were left intact over night. Gizzard shad were examined for gross external abnormalities and were kept for additional processing. Length and weight measurements were collected for each gizzard shad, and neoplasms were removed for additional

experimentation. Juvenile gizzard shad were collected with 0.8 cm seines at each of the three sites. Gizzard shad were also collected from Lake Carl Blackwell, Payne county, Oklahoma, and served as reference fish. Other incidental fish species were collected, however, our efforts were optimized for gizzard shad. Other species collected which did not exhibit external abnormalities were white bass (*Morone chrysops*), black crappie (*Pomoxis nigromaculatus*), river carpsucker (*Carpiodes carpio*), channel catfish (*Ictalurus punctatus*), flathead catfish (*Pylodictus olivaris*), and largemouth bass (*Micropterus salmoides*).

Tissue Processing for Reverse Transcriptase Analysis. Neoplastic tissues from gizzard shad collected from Lake of the Arbuckles and cranial, cutaneous, control tissues removed from gizzard shad collected from Lake Carl Blackwell were removed for reverse transcriptase (RT) analyses. The tissues were immediately frozen in liquid nitrogen and stored at -90°C for later analysis.

All procedural steps were completed at 4°C. Preliminary analysis of whole neoplasm homogenates revealed no evidence of RT activity. Therefore, to concentrate potential viral particles, multiple samples of neoplastic tissues were pooled, extracted, and subjected to density gradient fractionation. Control tissue (4.48 g wet weight, pooled from four fish) and GSN tissue (4.6 g wet weight, pooled from eight fish) were thawed and separately homogenized in 8 ml of TNES (10 m*M* Tris-HCl, pH 7.5, 100 m*M*

NaCl, 1.0 m*M* EDTA [ethylenediamine tetracetic acid], 5% sucrose). Homogenates were centrifuged for 30 min at 10,800 x g, and the supernatents were subjected to centrifugation at 100,000 x g for 2 hr. The pellets were resuspended in 150 μl of TNE (10 m*M* Tris-HCl, pH 7.5, 100 m*M* NaCl, 1.0 m*M* EDTA) containing 0.6 m*M* DTT (dithiothreitol), and the suspension was layered onto a 5 ml (15%-60% w/v) sucrose density gradient and centrifuged for 16 hr at 100,000 x g. Following centrifugation, 250 μl fractions were collected from the bottom of each tube (ISCO Model 1640 density gradient fractionator with a ISCO UA-5 absorbance/fluorescence detector). Refractive indices were determined, buoyant densities were calculated, and fractions were frozen at -90°C for subsequent RT analyses.

Reverse Transcriptase Analysis. The methods of Martineau *et al.* (19) were used in preliminary RT assays, but high background liquid scintillation counts with control and neoplasm samples obscured assay sensitivity. The second method described below yielded more consistent results with these tissues. The RT assays were performed as originally described by Tomley *et al.* (28), modified by Eaton and Kent, (16) and Schmale (per. com.) with other modifications as described below. Fractions were thawed and held on ice 30 min prior to assay. Aliquots of 35 μl from each fraction were mixed (at 2 min intervals) with 65 μl of RT cocktail [100 m*M* Tris, pH 8.3, 4 m*M* DTT, 50 m*M* KCl, 0.2% Nonidet P-40, 20 μg/ml poly rC·dG template

(Pharmacia, product # 27-7944-01)]; 5 μM dGTP (Pharmacia, product # 27-1870); 25 μCi/ml ^{3}[H]dGTP (New England Nuclear, specific activity 2.5 mCi/ml)] followed by addition of 10 μl of 10 mM $MnCl_2$. Reaction mixtures were combined and a 49 μl aliquot (T_0) was spotted onto GF/C filter paper (Whatman; 2.4 cm diameter) which had been presoaked in 100 mM sodium pyrophosphate ($Na_4P_2O_7 \cdot 10\ H_2O$) and allowed to dry overnight. Filter papers were immediately transferred into 10 ml of 10% trichloroacetic acid (TCA) and subjected to sequential 5 min washes in 5% TCA, 5% TCA/1% sodium dodecyl sulfate, and 95% ethanol with gentle shaking. The remainder of the reaction mixture was incubated at 28°C for 60 min and the process was repeated with a second 49 μl aliquot (T_{60}). Filter discs were dried completely and acid-precipitable counts-per-minute (CPM) were determined by liquid scintillation counting. Moloney Murine Leukemia Virus Reverse Transcriptase (MMLV-RT) was used as a positive control and distilled water served as a negative control. RT activity was determined by subtracting acid-precipitable CPM at T_0 from T_{60}. All fractions were examined in triplicate.

RESULTS

Epidemiology. After thirty-two months of collection, 795 adult gizzard shad were examined, and 168 of those individuals (22.3%) exhibited grossly visible GSN. Those fish caught ranged from 26.2 to 40.5 cm in

length, 206 g to 640 g in weight, and were, according to published data, assumed to be at least 2 to 3 years old (29). There was a significant difference (p = 0.05) in length and weight between gizzard shad with and without neoplasms (26). The non-neoplasm-bearing shad were shorter (34.27 cm, standard deviation 18.2 cm vs. 35.87 cm, standard deviation 16.2 cm) and weighed less (416.6 g, standard deviation 80.3 g vs. 434 g, standard deviation 67.9 g) (26). The juvenile fish collected (over 500) between the ages of 3 to 6 months did not exhibit observable neoplasms. No gizzard shad, juveniles or adults, (n = 212) collected from Lake Carl Blackwell exhibited neoplasms.

Reverse transcriptase Activity. No reverse transcriptase activity was detected in whole neoplasm homogenates or sucrose gradient fractions of pooled neoplasm homogenates (Fig. 2). Distilled water negative controls had counts of less than 50 CPM and MMLV-RT positive controls had counts of 110,000 CPM. When retroviruses have been isolated in malignant tissues of other fish species, peaks of reverse transcriptase activity were detected at fractions with buoyant densities of 1.16 to 1.18 g/ml (14, 20, 21).

DISCUSSION

This is the first report of peripheral nerve sheath epizootic occurring in a freshwater teleost. This case has similarities to the reports of PL in the chinook salmon (16) and neurofibromatosis in the bicolored damselfish (23,

30), because the gizzard shad were also collected in presumably pristine water. A comparable nerve sheath neoplasm in the bicolor damsel fish inhabiting coral reefs off the coast of Florida (30) is similar to the GSN found at Lake of the Arbuckles. Neoplasm incidence in the damselfish ranged from 0.4 to 23.8% (mean = 6.8%) of the sexually mature adults. A significantly larger portion of the gizzard shad population (22.3%) exhibit GSN and the occurrence of neoplasms is limited to Lake of the Arbuckles.

Nerve sheath neoplasms have been described in several marine species such as the genus, *Lutjans*, of the snapper family (31) and the slippery dick (*Halichoeres bivittatus*) (32). Reports of neurogenic neoplasms in freshwater and anadromous species have also been described in the coho salmon (*Onchorhynchus kitsuch*) (33, 34) and the goldfish (*Carassius auratus*), (35) and an African lungfish (*Protopterus annectens*) (36). Schwannoma was diagnosed in three rainbow smelt (*Osmerus mordax*) caught in estuaries at two sites in Nova Scotia (37). Schwannomas, like GSN are peripheral sheath nerve neoplasms. The etiology of rainbow smelt schwannoma is unknown. Morrison *et al.*(37) suggested, among other things, that anthropogenic pollutants and viral agents may lower the fish's immune response and allow for neoplasm formation.

Reverse transcriptase activity was not detected in whole neoplasm homogenates or in sucrose density gradient fractions of pooled neoplasm homogenates. In similar studies with fish models, RT activity was detected

in fractions with a buoyant density of 1.16 to 1.18 g/l (14, 16, 19). Two RT assay methods were used (20, 28). The RT assay methods of Martineau *et al.* (20) used a poly A template, but high background liquid scintillation counts with control and neoplastic samples obscured assay sensitivity. In an attempt to reduce background radiation counts, modifications of the methods of Tomley *et al.* (28) were tried using a poly C template in a pilot study. With GSN tissue fractions and control tissue fractions, the methods of Tomley *et al.* (28) appeared to be the most sensitive. According to Schmale, (per com.), RT assays involving fish tissues may differ in sensitivity depending on the ribonucleic template used (e.g. poly A versus poly C).

The negative reverse transcriptase activity data and the absence of observable viral particles in malignant tissue does not preclude the presence of another oncogenic virus. Studies are underway to determine if cell-free GSN extracts are transmissible into another species.

Located in a sparsely populated area, Lake of the Arbuckles is free of any apparent industrial discharge (26, 27). Examination of water and sediments from Lake of the Arbuckles via BTEX analysis and gas chromatography/mass spectrometry did not reveal any known cancer causing aromatic hydrocarbons (26, 27). Therefore, Lake of the Arbuckles appears to contain extremely low levels, if any, of contaminants associated with the petroleum industry. The absence of detectable levels of

contaminants, the lack of surface oil sheen, and the lack of characteristic odors are indicators of an unpolluted lake. However, other classes of compounds, such as metals, have been detected in association with fish neoplasms at other sites and are potential carcinogens to humans (38-40). Metal analyses are currently underway to determine the concentrations of cadmium, chromium, copper, nickel, and lead in water, sediment, and gizzard shad liver tissue.

In conclusion, neoplasm occurrence was stable over the last 9 months of collections, with an overall average of 22.3% of the adult gizzard shad exhibiting neoplasms. Sample sizes were larger and our collection efforts improved. The etiology of this disease remains unknown. Obvious causes, such as carcinogenic aromatic hydrocarbons and retroviral infection, were examined and do not appear linked to this disease (27). However, other carcinogenic agents, such as heavy metals and other infectious organisms, cannot be excluded. Neurogenic neoplasms have been induced in rats (41) and several fish species (42, 43) when exposed to strongly carcinogenic compounds such as methylazoxymethanol.

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Table 1. Neoplasm frequency in gizzard shad collected from Lake of the Arbuckles, Murray county, Oklahoma.

[a]Date of collection	Number of Shad caught	Number of shad with neoplasms	Percent of shad with neoplasms
8-7-91	105	15	14.3
12-5-91	11	1	9.1
4-24-92	80	5	6.3
8-21-92	69	7	10.1
9-12-92	11	3	27.3
10-11-92	16	9	56.3
1-7-93	109	30	27.5
3-28-93	270	67	24.8
6-29-93	85	23	27.1
9-25-93	39	8	20.5
TOTALS	795	161	22.3[b]

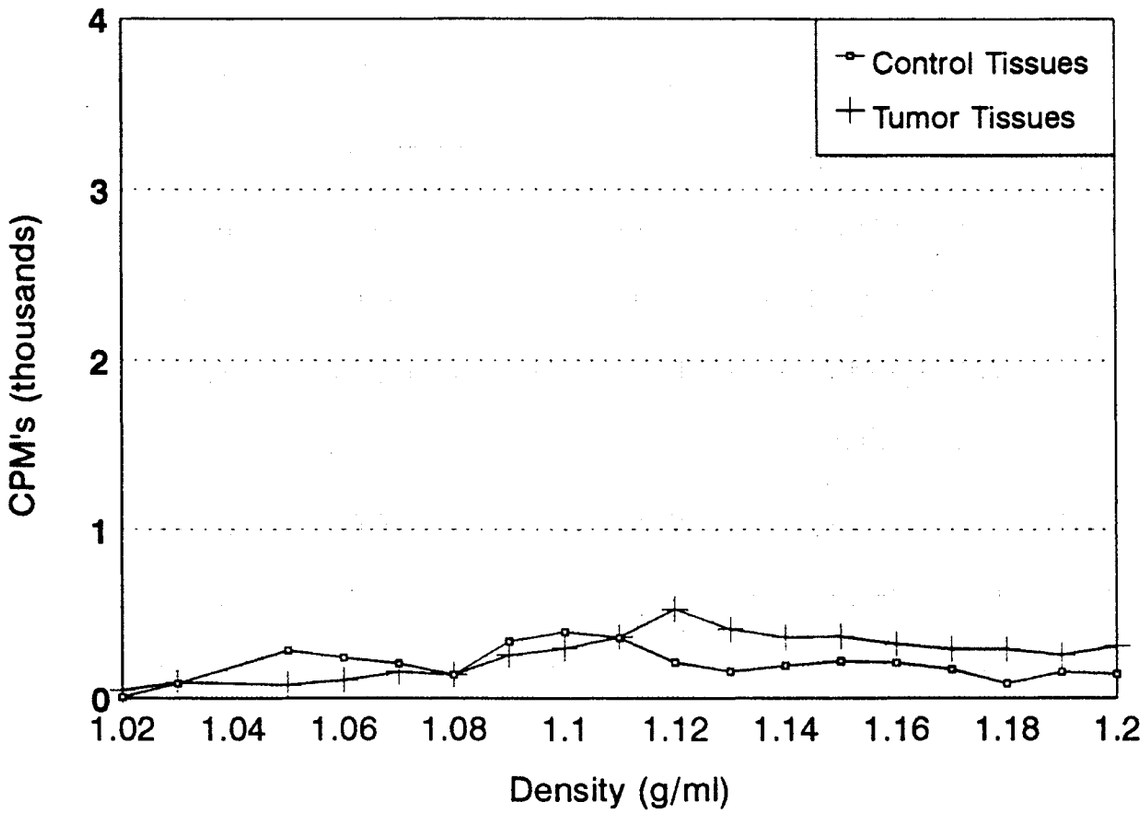
[a]Collections from 8/91 to 3/93 were previously presented (26).

[b]Calculated value is the mean of the individual sampling means.

Figure 1. Map of sampling sites. Lines from continental United States map indicate approximate locations of the lakes in the State of Oklahoma (shown in black). Sampling locations at Lake of the Arbuckles, Murray county, are indicated by letters A-C. Sampling locations at the reference site, Lake Carl Blackwell, Payne county, are designated by letters D and E. (Scale is 1 mile ————).


Guy Sandy Site
A
Rock Creek Site
B
Buckhorn Site
C
Lake of the Arbuckles
N
N
Site 2
E
D
D
Site 1
Lake Carl Blackwell

Figure 2. Analysis of neoplastic and control gizzard shad tissue homogenates for reverse transcriptase activity.



CHAPTER III

ETIOLOGICAL STUDIES OF SUBCUTANEOUS NEOPLASMS IN FERAL GIZZARD SHAD, *DOROSOMA CEPEDIANUM*

ABSTRACT

The etiology of subcutaneous neoplasms (gizzard shad neoplasms) in feral gizzard shad (*Dorosoma cepedianum*) was investigated. The objectives of this study were to (1) survey the incidence and seasonality of neoplasms; (2) measure the concentration of heavy metals in water, sediment, and gizzard shad liver tissue collected from Lake of the Arbuckles and the reference site, Lake Carl Blackwell; and (3) evaluate transmissibility of gizzard shad neoplasms into healthy rainbow trout (*Oncorhynchus mykiss*). Field collections of adult and juvenile gizzard shad were conducted to determine epidemiology including seasonality of neoplasms and site specificity. Water, sediment, and shad livers from Lake of the Arbuckles and Lake Carl Blackwell, Oklahoma, were analyzed for total recoverable metals, namely cadmium, chromium, copper, nickel, and lead, by graphite furnace atomic absorption. Chromium, copper, and nickel were found in the water from both lake samples in concentrations of >1 to 8.6 μg/L. Low

concentrations (>1 to 13.6 μg/g wet weight) of all the metals were found in the sediment and liver tissue. To determine if a non-bacterial infectious agent was responsible for neoplasm formation, cell-free homogenates were injected into healthy rainbow trout. Neoplasms were not transmitted into rainbow trout.

INTRODUCTION

The occurrence of epizootic neoplasms among teleost fishes has been widely documented throughout North America [1]. Three factors have been associated with neoplasms in feral fish species, namely exposure to carcinogens, oncogenic viral infection, and genetic predisposition.

Some epizootics of cancer in fish appear linked to chemical contamination. Upon histopathological examination, 100% (20 out of 20) of the saugers, *Stizostedion canadense*, collected from Torch Lake, MI, in 1979, exhibited liver neoplasms [2]. Over 27% (3 out of 11) of the walleye, *Stizostedion vitreum*, another closely related species, also collected from Torch Lake exhibited hepatocellular carcinomas and dermal ossifying fibromas. Intensive copper mining of the periphery of Torch Lake led to heavy metal contamination of the adjacent water. It was estimated that as much as 20% of Torch Lake's original volume was filled with copper mining wastes during the most productive mining period. Later, the deposited copper tailings were dredged from the sediments at Torch Lake at the end of the mining era. The deposition and subsequent dredging of the mining wastes caused water quality to decline. Copper, and other metals, such as cadmium, nickel, zinc, and lead, were detectable within the water column of Torch Lake, and elevated levels of copper, lead, chromium, cadmium, and zinc were distributed throughout the different layers of sediment from Torch Lake. Metal levels in the sediments ranged from 300 to 1,700 mg/L for

copper, 80 to 270 mg/L for lead, 30 to 40 mg/L for chromium, less than 2 mg/L of cadmium, and ≤180 mg/L of zinc. Black et al. [2] suggested that either high copper concentrations, other compounds associated with copper mining such as silica dust or asbestos, or metals combining to form new carcinogenic compounds may have synergistically acted to cause neoplasm formation.

Anthropogenic pollutants, however, do not appear to be linked to all cancer epizootics in fish. Oncogenic viruses have been implicated in several epizootics such as hyperplastic skin lesions in the northern pike (*Esox lucius*) [3], and papillomatous neoplasms of the common carp, (*Cyprinus carpio*) [4].

The pike herpesvirus is responsible for hyperplastic skin lesions in 1 to 7% of the northern pike (*Esox lucius*) collected from Saskatchewan and Manitoba, Canada [3]. Lesions were most prevalent in adult pike during spring spawning. The virus is suspected to be present in other geographic locations.

An oncogenic herpesvirus was responsible for neoplasm formation in 55% of common carp (*Cyprinis carpio*) immersed in water infected with Cyprinid herpesvirus-1 (CHV) within 5 to 6 months of exposure [4]. Three other species of cyprinids were used in transmissibility studies; however, carp appeared especially susceptible to CHV and papilloma formation. It appeared that proliferation of the infective virus in the epidermal tissue after inoculation with CHV may be arrested or initiated, contingent upon seasonal

water temperature variation.

In some epizootics of cancer in fish, occurrence was seasonal. Two such cases are the lymphosarcoma of the northern pike (*Esox lucius*) [5] and the dermal sarcoma of the walleye (*Stizostedion vitreum*) [6].

Lymphosarcomas were present in 20.9% of the adult northern pike collected throughout North America [7]. Tumor incidences were highest during early spring, regressed during the summer, and returned later in the fall [5]. According to Sonstegard [5], transmission occurred during spawning, and that decreased tumor incidence may have been caused by death of tumor-bearing fish or spontaneous regression of tumors. Transmission of the disease by injection of malignant cell-free filtrates into healthy adult northern pike was successful [8]. Lymphosarcomas appear to be retrovirally induced [7]. Reverse transcriptase activity, a diagnostic character of retroviral infection, was detected in malignant tissues.

Greater than 27% of the adult walleye population collected from Oneida Lake, NY, in 1986, exhibited benign dermal sarcomas [6]. Tumors were most prevalent during early spring and late fall. Bowser et al. [8] speculated that suppression of the immune system, due to decreased water temperature, may have allowed viral infection to induce tumors. A retrovirus has been associated with these neoplasms [9].

In this paper, the etiology of the pigmented subcutaneous spindle cell neoplasms [10], referred to as gizzard shad neoplasms (GSN), was

investigated. Feral gizzard shad, *Dorosoma cepedianum*, collected in Lake of the Arbuckles exhibited raised, darkened, neoplasms. We have surveyed the epidemiology of GSN and have examined two factors which have been linked to epizootics of cancer, namely anthropogenic contaminants and oncogenic viral infection. We report the results of seasonality and prevalence of neoplasms, analysis of heavy metals from the examination of water, sediment, and shad liver tissue from Lake of the Arbuckles and Lake Carl Blackwell, and transmissibility of these neoplasms into a heterospecific species.

To determine if GSN were seasonal, we collected fish at 3-month intervals and expanded our collection to 990 individuals over a three-year period. Three sites in Lake of the Arbuckles were commonly sampled to determine if the disease was site specific.

In view of the absence of aromatic hydrocarbons in the water and sediments of Lake of the Arbuckles [10], we wanted to determine if heavy metals were present in sufficient concentrations to induce carcinogenesis. Water, sediment, and gizzard shad livers from Lake of the Arbuckles and Lake Carl Blackwell were examined for the presence of cadmium, chromium, copper, nickel, and lead by graphite furnace atomic absorption (GFAA). These metals were chosen because they have been found in association with feral fish neoplasms at other sites, exhibited toxic effects, and have been implicated as possible carcinogens in humans [2, 11-15].

To determine if a non-bacterial infectious agent (i.e. a virus) was responsible for neoplasm formation, healthy rainbow trout were injected with cell-free extracts of GSN for transmissibility studies. If these attempts were successful, a laboratory model could be established for further study of this disease.

MATERIALS AND METHODS

Study sites

Lake of the Arbuckles is a man-made lake located in south central Oklahoma (Fig. 3). It was completed in 1967 to serve as a source of water and a recreational lake for the two neighboring towns of Sulfur and Davis with populations of 5,500 and 2,800, respectively. It is formed at the confluence of the Guy Sandy, Rock, and Buckhorn creeks, is encompassed by 58 km of shoreline, and has a mean depth of 9.4 m.

Upon completion in 1938, Lake Carl Blackwell, a man-made impoundment located 14 km west of Stillwater in north central Oklahoma, served as a flood control system and as a recreational lake for Payne county (Fig. 3). Stillwater Creek was dammed as part of the Federal Government Land Utilization Project. Lake Carl Blackwell has a mean depth of 4.93 m and is encompassed by 88.5 km of shoreline.

Fish collection

Adult gizzard shad (assumed 2 to 5 years old, 310 to 490 mm in length) were collected with gill nets as previously described [10]. Fish were examined for gross external abnormalities, and length and weight measurements were made for each gizzard shad. Neoplasms were removed for tissue processing. Juvenile gizzard shad were collected with 0.8 cm mesh beach seines at each of the sites. Gizzard shad were collected in a similar manner from Lake Carl Blackwell to serve as reference fish.

Tissue processing

Gizzard shad neoplasm tissue was removed from Lake of the Arbuckles gizzard shad immediately upon collection and frozen in liquid nitrogen for transmissibility studies. All tissues were transported to the laboratory and subsequently stored at -90°C.

Livers from neoplasm bearing (n = 18) and non-neoplasm bearing gizzard shad (n = 18) from Lake of the Arbuckles and reference fish from Lake Carl Blackwell (n = 12) were immediately removed and placed in acid-rinsed, 15-ml polypropylene centrifuge tubes. Livers were frozen in liquid nitrogen and were stored at -90°C prior to heavy metals analyses.

Water, sediment and liver preparation for metal analyses

Three water samples were collected from each site approximately 61

cm below the surface in acid-rinsed, polypropylene containers. Field blanks were used to assess transportation contamination. Polypropylene containers filled with distilled-deionized water for field blanks were manipulated in the same manner as the water samples. Samples and blanks were placed on ice, transported back to the laboratory, acidified to pH < 2 (~ 1.5 ml, TraceMetal Grade nitric acid, Fisher Scientific, Pittsburgh, PA) and were subsequently acid digested. Environmental Protection Agency (EPA) acid digestion Method 3020 (as outlined in SW-846 protocol for determination of total recoverable metals by GFAA) was followed for the preparation of water samples [15].

Three sediment samples from each site were collected with an acid-rinsed Ekman dredge and transferred to an acid-rinsed polypropylene bucket. The three dredges were then mixed for a composite sample and were placed in acid-rinsed, polypropylene containers. Field blanks of distilled-deionized water were treated in a similar fashion. Samples were placed on ice, transported to the laboratory, and stored in total darkness at -20°C until acid digestion.

Modifications of acid digestion Method 3050 (as outlined in SW-846 EPA protocol for total metals determination by GFAA) was employed for sediment and liver sample preparation [16]. A prolonged nitric acid reflux of 12 h was used to oxidize organic material. Duplicate samples were digested for each water and sediment sample, and one duplicate per each six livers

was also digested.

A Perkin-Elmer 5000 atomic absorption spectrophotometer equipped with a deuterium arc lamp background corrector, a HGA 500 heated graphite atomization apparatus, and an AS-40 autosampler was used for analyses of cadmium, chromium, copper, nickel, and lead. Three or more in-house standards of commercially available certified stock solutions (Metal Reference Solution, Fisher Scientific, Pittsburgh, PA) and EPA reference standards were analyzed with each set of samples. Concentrations were calculated using linear regression. Recovery of metal spikes added to water, sediment, or liver tissue was 90 to 104 percent. All duplicate sample concentrations were within ± 5% of one another or were re-digested and re-analyzed.

Statistics

Concentrations were reported as μg/L for water samples and μg/g wet weight for liver and sediment samples. For analyses of the concentrations of heavy metals, all data were initially tested for normality and homogeneity of variance. Metal concentrations for the liver samples were not normally distributed, and appropriate nonparametric statistics were performed. Metal concentrations for the liver data were ranked by concentration without regard to grouping using the Wilson-Shapiro method [17]. An ANOVA was performed on the ranks of the liver metal concentrations, and the LSD

procedure was used to assess differences among the mean population concentrations [17]. Water and sediment data were found to be normally distributed, and *t*-tests were performed to distinguish differences between site concentration means. The level of significance for all metal concentration data analyses was $p = 0.05$.

Transmissibility studies

Gizzard shad are not a hardy species and are difficult to maintain in a laboratory for extended periods of time (e.g. 6 months to 1 year), and therefore a more suitable species was chosen for transmissibility studies. Healthy rainbow trout (*Oncorhynchus mykiss*) (length 147 mm to 190 mm) were maintained in two Living Stream (Frigid Units, Inc.Toledo, OH) recirculating raceway aquaria at 13 to 15°C with 12 h cycles of light and darkness two months prior to the study. Modifications of the method of Martineau et al. [19] were used to evaluate the transmissibility of GSN into rainbow trout. Gizzard shad neoplasm tissue (1.4 g) from eight shad from Lake of the Arbuckles was pooled for this experiment. The following steps were conducted at 4°C: pooled GSN tissues were minced with scissors, ground in 5 ml of phosphate buffered saline (PBS, pH 6.8) with a tissue grinder, and further disaggregated by homogenization. The GSN homogenate was sonicated for 15 min to disrupt cells, and the suspension was passed through a 2 μm syringe filter to remove whole cells and cell

debris. Prior to injection, trout were anesthetized in 150 mg of tricaine methanesulfonate (Crescent Research Chemicals, Phoenix AZ) dissolved in 3 liters of tank water. Experimental fish (n = 17) were given three 50 µl injections, 2 subcutaneous anterior to the dorsal fin and 1 intraperitoneal anterior to the pelvic fin. Control fish (n = 17) were injected with 50 µl of PBS at the same three injection sites. Experimental fish were marked by removal of the adipose fin, and experimental and control trout were reared together (1:1) in two tanks to avoid tank bias. Trout were observed daily to assess health and were fed four times per day a diet of Purina® Trout Chow brand commercial fish food. Aquaria conditions such as temperature, pH, and ammonia levels were also monitored daily.

The transmissibility study was terminated after 288 days. All fish were sacrificed, and gross necropsies were performed. Length and weight measurements and sex were recorded. Tissues examined included epidermis, gills, liver, intestines, and muscle. Suspicious tissues were preserved in neutral buffered formalin for histopathological examination.

RESULTS

Seasonality of neoplasms

A large cumulative sample of gizzard shad from Lake of the Arbuckles at seasonal intervals was collected (Table 2). Neoplasm incidences ranged from 6.3 to 56.3%. Gizzard shad with s were collected at all three sites,

however the majority were collected at the Guy Sandy site. Over 500 juvenile gizzard shad (> 1 year old) from Lake of the Arbuckles were examined and none exhibited grossly observable neoplasms. Neoplasms did not appear to be seasonal when similar sample sizes were compared. Gizzard shad (n = 12 adults, n = 200 juveniles) collected from Lake Carl Blackwell did not exhibit grossly observable neoplasms.

Metal concentrations in water, sediment, and liver tissue

Statistical differences at p = 0.05 level were denoted with asterisks for the data in Tables 3-5. Specific differences for the metal concentrations were not considered biologically significant because the concentrations of these metals were very low (i.e. μg/L and μg/g range) and did not exceed State of Oklahoma, EPA, or Food and Drug Administration action levels (Tables 3-5).

The mean concentrations of chromium in the water from Lake Carl Blackwell was eight times higher than water from Lake of the Arbuckles (Table 3). Copper concentrations in the water from Lake of the Arbuckles were nearly ten-fold higher and ranged from 3.88 to 6.49 μg/L, whereas samples taken from Lake Carl Blackwell had concentrations which ranged from 0 to 0.5 μg/L. Detectable levels of lead were observed in water samples from Lake of the Arbuckles and concentrations ranged from 0.74 to 1.62 μg/L, but the levels were below detection limits of 50 ng/L at Lake Carl

Blackwell. Cadmium was below detection limits of 3 ng/L in water samples from both lakes.

Metal sediment concentrations (μg/g wet weight) were somewhat similar between sites and lakes. Mean combined-site sediment concentrations of lead were four times higher at Lake of the Arbuckles than at Lake Carl Blackwell (1.33 vs. 5.33 μg/g) (Table 4).

Liver concentrations of metals were reported as μg/g wet weight. Differences among populations of gizzard shad were inconsistent and no apparent trend was obvious. Non-neoplasm-bearing shad collected from Lake of the Arbuckles had higher concentrations of chromium (0.43 μg/g vs. 0.19 μg/g), nickel (2.32 μg/g vs. 1.47 μg/g), and lead (0.46 μg/g vs. 0.27 μg/g) in their livers than did neoplasm-bearing shad from the same site (Table 5). Nearly twice as much copper was found in the livers of shad collected from Lake of the Arbuckles than in the livers of the shad from Lake Carl Blackwell (4.53 and 4.13 μg/g vs. 2.61μg/g). Cadmium concentrations in the livers of shad from Lake Carl Blackwell were two times higher than shad from Lake of the Arbuckles (0.16 μg/g vs. 0.06 and 0.07 μg/g). The mean concentration of nickel in the livers of neoplasm and non-noplasm-bearing shad (1.47 and 2.32 μg/g vs. 0.17 μg/g) was more than 11 times higher than nickel concentrations in the livers of shad from Lake Carl Blackwell. With the exception of cadmium, metal concentrations in the livers were higher in shad from Lake of the Arbuckles, without respect to the

neoplasm or non-neoplasm-bearing condition.

Transmissibility studies

After 288 days, post injection necropsies did not reveal any external or internal lesions. Tissues examined appeared to be healthy, except for white spots on 14.7% (5 out of 34) of the kidneys, of one control trout and four experimental trout. Histopathological examination of those kidneys bearing white spots did not reveal any neoplastic cells.

DISCUSSION

This epizootic of GSN is unique with respect to its high prevalence, species affected, and geographic location. Epizootics of cancer in fish often have three factors linked to their etiology, namely exposure to carcinogens, oncogenic viral infection, and, genetic predisposition. In this study, we examined seasonality of neoplasms, environmental carcinogenic metal exposure, and oncogenic viral infection.

No evidence of seasonality similar to that seen in the lymphosarcomas of the northern pike (*Esox lucius*) [5] and the dermal sarcomas in the walleye (*Stizostedion vitreum vitreum*) [6] was observed in Lake of the Arbuckles gizzard shad exhibiting GSN. Neoplasm incidence varied much less from January, 1993, to March, 1994, vs. prior collections, possibly due to better collection techniques.

Although more gizzard shad were collected at the Guy Sandy site, shad bearing GSN were collected at all three sites. This difference may be due to habitat preference rather than clustering ofneoplasm-bearing individuals. The Guy Sandy site is shallower and faces the north, so that wind action can distribute sediments and plankton throughout the water column.

None of the metal concentrations observed exceeded EPA water quality criteria or Food and Drug Administration limits of metals in fish tissues. No inference is made that the metal concentrations observed were biologically significant because the levels were so low. Oklahoma Geologic Survey data indicated that zinc and lead mines adjacent to streams feeding into Lake of the Arbuckles may be a source of metals [20]. One might conclude that the metals were present in minute quantities at Lake of the Arbuckles and at Lake Carl Blackwell, and that the gizzard shad were accumulating the metals in their livers. In several studies, metals (e.g. cadmium, lead, and copper) tended to accumulate at concentrations nearly ten times higher in the liver than in muscle tissue or other organs [21-23]. The liver is one site of detoxification which appears to serve as a reservoir for some metals in fish. The concentrations of metals present at either lake were not sufficiently high to be considered carcinogenic or promote neoplasm formation in the gizzard shad. Metal content in the livers were not consistent with epidemiology. Non-neoplasm-bearing shad accumulated

particularly more of four of the metals in their livers than did neoplasm-bearing shad.

The absence of transmissed GSN into rainbow trout, combined with negative reverse transcriptase activity data and lack of C-type viral particles in neoplastic tissues examined by elecron microscopy, strongly suggested that a retrovirus was not the infective agent. This does not preclude the presence of other infectious viruses which may be responsible for GSN formation. Oncogenic viruses such as the papillomavirus, the adenovirus, and the herpesvirus have been known to cause cancer in humans and other animals [24-26].

The inability to transmiss GSN into healthy rainbow trout may have been due to differences between species. If an infectious virus were present in the cell-free GSN extracts, the rainbow trout may not be a suitable host or may possess immune system characteristics that make them resistant to the virus.

Some viruses, such as the herpesvirus responsible for the Lucké tumor of the leopard frog, *Rana pipiens*, require infected frogs to hibernate at temperatures below 10°C before the virus becomes active [28]. Only after the cold treatment can the extracts from tumor-bearing frogs produce tumors in healthy individuals. Naegele and Granoff [28] suggested that a "helper virus" or some unknown agent may be responsible for activation of the Lucké Herpes Virus (LHV) that leads to tumor formation in healthy frogs.

In addition, the LHV is limited to frogs in a specific locality. The unique specificity of the LHV may also be similar to a viral agent responsible for the GSN in gizzard shad from Lake of the Arbuckles. Viral particles were not observed in GSN from Lake of the Arbuckles shad under extensive electron microscopy, but that observation does preclude the presence of a virus. Likewise, viral particles could not be detected in naturally occurring LHV tumor tissue examined by electron microscopy until the malignant tissues were subjected to cold treatment.

Other possible etiology of GSN may include other classes of anthropogenic pollutants such as radon or halogenated pesticides. Other cases of GSN have been reported in gizzard shad collected at Lake Texoma, Oklahoma [Jimmie Pigg, pers. com.]. Both Lake of the Arbuckles and Lake Texoma are within the same river drainage and could provide evidence to suggest anthropogenic causality. A carcinogen may be introduced into tributary streams above Lake of the Arbuckles and Lake Texoma and adsorb onto sediment particles or plankton. The adsorbed contaminant could then be distributed throughout the river drainage.

In addition, a hemangiopericytoma was found on one white bass, *Morone chrysops,* residing in Lake of the Arbuckles (Hawkins et al., in prep). This type of neoplasm has never been seen in a white bass and may also be suggestive of an anthropogenic cause. A larger sample size of the white bass is needed to determine extent and significance of this neoplasm. The

origin is similar to the GSN and could also be initiated by exposure to carcinogens. The white bass is a predator of the gizzard shad and could pose a argument for biomagnification of an unknown anthropogenic contaminant within the food chain at Lake of the Arbuckles.

Gizzard shad are planktivores and may be exposed to anthropogenic contaminants adsorbed to phytoplankton and zooplankton or pollutants sequestered in the sediments [29-31]. Forage fish like gizzard shad are not as economically important to fishermen as sport species. Therefore, few site characterization investigations have used gizzard shad as a specimen for study. The gizzard shad's place in the food chain of an aquatic ecosystem should make them an ideal study species for bioaccumulation and biomagnification studies.

A well documented example of inherited cancer is the melanomas of the offspring of hybrid crosses of platyfish (*Xiphophorus maculatus*) and swordtails (*X. helleri*) [32]. Melanoma formation is the result of a sex-linked gene present in hybrid progeny that do not possess an autosomal locus that serves as a tumor suppressor gene [33]. The Lake of the Arbuckles shad population may also be genetically predisposed to GSN formation. The population may be inbred, thus increasing the chances of either hemizygous or homozygous loss of a tumor suppressor gene or a mutation that causes activation of an oncogene.

The etiology of GSN is not known. The most common causative

agents in other epizootics of cancer in fish have been examined in this report, namely presence of carcinogens at the affected site, and viral infection. No evidence of exposure to potentially carcinogenic compounds or retroviral infection have been found in gizzard shad from Lake of the Arbuckles. Therefore, data combined from this report and a previous study suggest that Lake of the Arbuckles is a relatively clean lake and free of obvious pollutants [10].

In summary, 22.1% of the population of gizzard shad residing in Lake of the Arbuckles exhibit neoplasms. No seasonality of neoplasms were observed. Metal concentrations observed in Lake of the Arbuckles gizzard shad livers, sediment, and water were too low to warrant causality of GSN formation. Rainbow trout did not develop GSN after injection of cell-free malignant tissue homogenates. More studies are needed to determine the geographic distribution, population and community effect, and genetic predisposition of this disease as well as extensive detailed chemical analyses of water, sediment, and fish tissue for the presence of other anthropogenic compounds.

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Table 2. Neoplasm frequency in gizzard shad collected from Lake of the Arbuckles, Murray county, Oklahoma.

[a]Date of collection	Number of Shad caught	Number of shad with neoplasms	Percent of shad with neoplasms
8-7-91	105	15	14.3
12-5-91	11	1	9.1
4-24-92	80	5	6.3
8-21-92	69	7	10.1
9-12-92	11	3	27.3
10-11-92	16	9	56.3
1-7-93	109	30	27.5
3-28-93	270	67	24.8
6-29-93	85	23	27.1
9-25-93	39	8	20.5
12-19-93	59	13	22.0
3-27-94	136	27	19.8
TOTALS	990	208	[b]22.1

[a]Collections from 8/91 to 9/93 were discussed in Ostrander et al., 1994 and were presented for examination of seasonality of neoplasms.

[b]Calculated value is the mean of the individual sampling means.

Table 3. Mean concentration (μg/L) and standard deviation of five metals found in water samples collected from Lake of the Arbuckles and Lake Carl Blackwell, Oklahoma. Each site concentration is the mean of duplicate samples.

	Element				
Site	Cd	Cr	Cu	Ni	Pb
Lake of the Arbuckles					
Guy Sandy	BDL	1.38	3.88*	4.11	0.74*
SD	N/A	0.0001	0.062	0.001	0.001
Rock Creek	BDL	1.40	6.49*	4.43	1.27*
SD	N/A	0.034	0.001	0.001	0.001
Buckhorn Creek	BDL	1.26	3.89*	5.39	1.62*
SD	N/A	0.034	0.062	0.001	0.001
Lake Carl Blackwell					
Site 1	BDL	8.30*	BDL	4.75	BDL
SD	N/A	0.033	N/A	0.001	N/A
Site 2	BDL	8.55*	0.50	2.50	BDL
SD	N/A	0.034	0.001	0.001	N/A

*Significant at (p = 0.05), BDL (Below detection limits)

Table 4. Mean concentration (μg/g wet weight) and standard deviation of five metals found in sediment samples collected from Lake of the Arbuckles and Lake Carl Blackwell, Oklahoma. Each site concentration is the mean of duplicate samples.

	Element				
Site	Cd	Cr	Cu	Ni	Pb
Lake of the Arbuckles					
Guy Sandy	0.09	7.57	6.15	6.35	5.66*
SD	0.002	0.052	0.042	0.044	0.040
Rock Creek	0.16	10.40	5.96	7.07	6.13*
SD	0.0006	0.021	0.012	0.015	0.013
Buckhorn Creek	0.25	13.60	7.01	7.06	4.19*
SD	0.001	0.322	0.037	0.037	0.022
Lake Carl Blackwell.					
Site 1	0.08	12.57	7.11	5.81	0.97
SD	0.0002	0.136	0.099	0.192	.035
Site 2	0.24	6.32	4.05	5.80	1.69
SD	0.0004	0.009	0.008	0.192	0.019

*Significant at (p = 0.05)

Table 4. Mean concentration ($\mu g/g$ wet weight) and standard deviation of five metals found in sediment samples collected from Lake of the Arbuckles and Lake Carl Blackwell, Oklahoma. Each site concentration is the mean of duplicate samples.

	Element				
Site	Cd	Cr	Cu	Ni	Pb
Lake of the Arbuckles					
Guy Sandy	0.09	7.57	6.15	6.35	5.66*
SD	0.002	0.052	0.042	0.044	0.040
Rock Creek	0.16	10.40	5.96	7.07	6.13*
SD	0.0006	0.021	0.012	0.015	0.013
Buckhorn Creek	0.25	13.60	7.01	7.06	4.19*
SD	0.001	0.322	0.037	0.037	0.022
Lake Carl Blackwell.					
Site 1	0.08	12.57	7.11	5.81	0.97
SD	0.0002	0.136	0.099	0.192	.035
Site 2	0.24	6.32	4.05	5.80	1.69
SD	0.0004	0.009	0.008	0.192	0.019

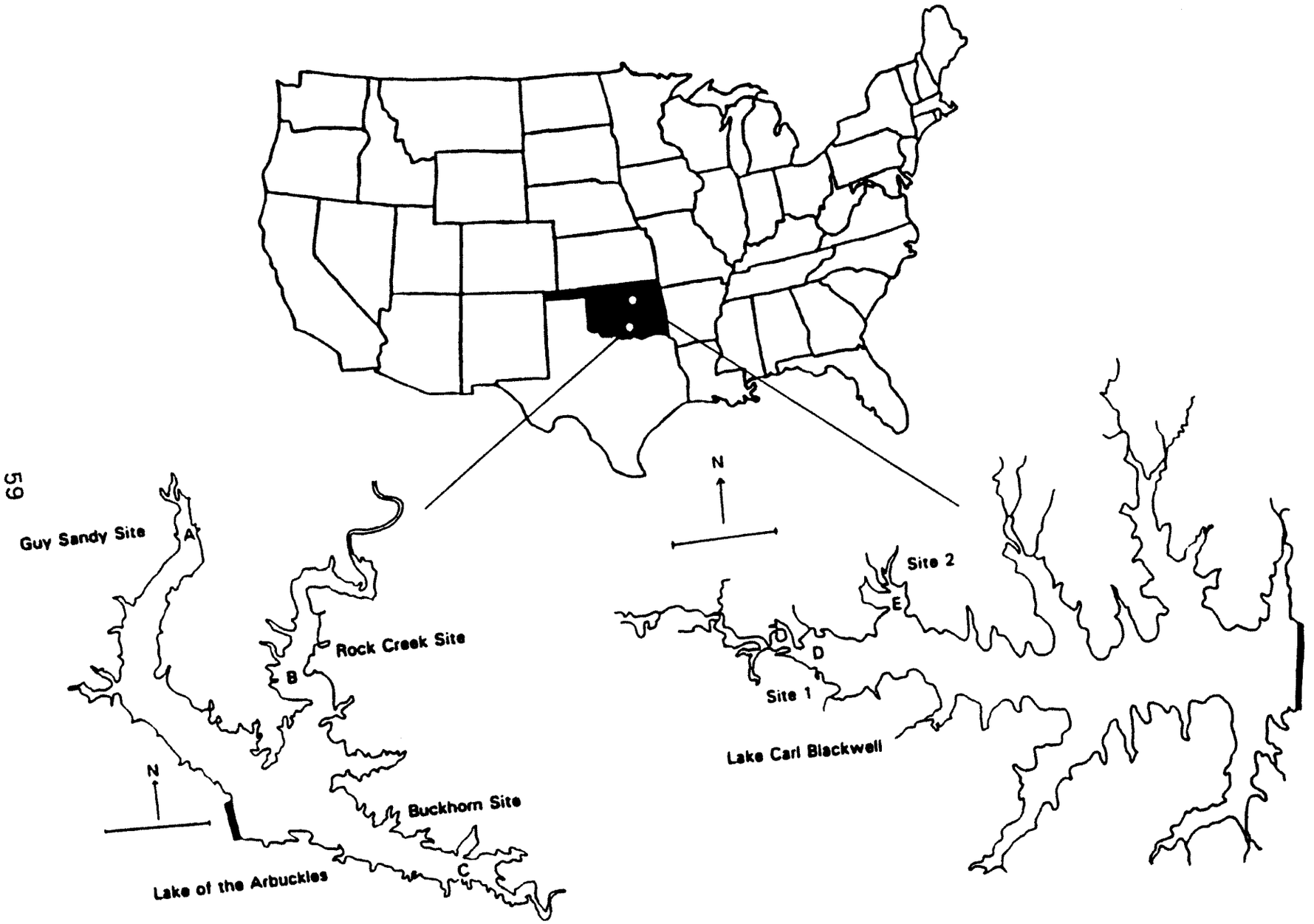
*Significant at (p = 0.05)

Table 5. Mean concentration (μg/g wet weight) and range of five metals in the livers of adult gizzard collected from Lake of the Arbuckles and Lake Carl Blackwell Oklahoma.

Site and type	Element Cd	Cr	Cu	Ni	Pb
Lake of the Arbuckles n = 18					
Neoplasm-bearing shad	0.07	0.19	4.53*	1.47	*0.27
Range	(0-0.13)	(0-1.04)	(1.73-11.65)	(0.06-5.27)	(0-1.78)
Non-neoplasm-bearing shad	0.06	0.43*	4.13*	2.32*	0.46*
Range	(0.01-0.13)	(0-1.19)	(1.31-7.79)	(0.03-6.71)	(0.18-0.92)
Lake Carl Blackwell n = 12					
Reference	0.16*	0.13	2.61	0.17	0.11
Range	(0.12-0.27)	(0-0.44)	(1.75-4.61)	(0-0.50)	(0-0.21)

*Significant at (p = 0.05)

Figure 1. Map of sampling sites. Lines from continental United States map indicate approximate locations of the lakes in the State of Oklahoma (shown in black). Sampling locations at Lake of the Arbuckles, Murray county, are indicated by letters A-C. Sampling locations at the reference site, Lake Carl Blackwell, Payne county, are designated by letters D and E. (Scale is 1 mile ————).


Guy Sandy Site
A
Rock Creek Site
B
Buckhorn Site
C
N
Lake of the Arbuckles
Site 2
E
D
Site 1
N
Lake Carl Blackwell

VITA

ANN DURHAM JACOBS

Candidate for the Degree of

Master of Science

Thesis: FURTHER STUDIES OF PIGMENTED SUBCUTANEOUS SPINDLE CELL NEOPLASMS IN GIZZARD SHAD, *DOROSOMA CEPEDIANUM*, RESIDING IN LAKE OF THE ARBUCKLES, MURRAY COUNTY, OKLAHOMA

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