

Diagnostic criteria for proliferative hepatic lesions in brown bullhead *Ameiurus nebulosus*

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ABSTRACT: Brown bullhead *Ameiurus nebulosus* is used as indicator species for contaminant effects at areas of concern (AOC) in the Great Lakes and other areas. One of the beneficial use impairments at numerous AOC is 'fish tumors and other deformities'. An impairment occurs when the prevalence of fish tumors and other deformities exceeds those at unimpacted or control sites or when survey data confirm the presence of neoplastic or preneoplastic liver lesions in bullhead or white sucker *Catostomus commersonii*. Numerous surveys have been conducted over the years assessing neoplasia in these fishes, both liver and skin tumors. However, a major problem in comparing the results has been a lack of consistent criteria for evaluating histological changes in bullhead livers. As individual AOC develop and implement remedial action plans, realistic and attainable delisting targets need to be specified. For this to occur and be consistent from site to site there must be standardization of the criteria being used to evaluate specific impairments. In this report, specific diagnostic criteria are provided for both non-neoplastic and neoplastic proliferative hepatocellular and biliary lesions. These criteria should assist fish pathologists in describing and categorizing proliferative liver lesions from brown bullhead.

KEY WORDS: Brown bullhead · Liver · Neoplasia · Proliferative lesions · Diagnostic criteria

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INTRODUCTION

The International Joint Commission (IJC), comprised of American and Canadian officials, was formed in 1909 to assist their governments in finding solutions to the problems facing the waters bordering the United States and Canada. The Great Lakes Water Quality Agreement, in which the United States and Canada agreed to restore and preserve the biological, physical and chemical integrity of the Great Lakes Basin Ecosystem, was first signed in 1972. In 1987 a protocol was signed by both governments which defined Great Lakes areas of concern (AOC) as 'geographic areas that fail to meet the general or specific objectives of the agreement where such failure has caused or is likely to cause impairment of beneficial use of the area's ability to support aquatic life'. The US and Canadian governments identified 43 such areas; 26 in the US, 12 in

Canada, and 5 shared between the US and Canada on connecting river systems. The Great Lakes Water Quality Agreement, as amended via the 1987 protocol, directs the 2 federal governments to cooperate with state and provincial governments to develop and implement remedial action plans (RAPs) for each AOC. The protocol also called for reports on restorative progress and for the IJC to review RAPs proposed by the 43 AOC. To date, 2 AOC (Collingwood Harbor and Severn Sound) have been delisted (IJC 1987) and Presque Isle Bay has been designated an area of recovery.

So far, 14 beneficial use impairments have been identified and the various AOC have different combinations of these impairments. One impairment, listed as 'fish tumors and other deformities' is defined as occurring when 'the incidence rate of fish tumors and other deformities exceeds rates at unimpacted or control sites or when survey data confirm the presence of

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neoplastic or preneoplastic liver tumors in bullhead or suckers' (IJC 1989; www.epa.gov/lakeerie/buia/reports). Deformities are defined as twisted, missing, forked or bulging body parts including fins, barbels, abdomen or skeleton. Currently, 16 of the 41 AOC have the presence of tumors or other deformities listed as one of their beneficial use impairments.

A key indicator species for AOC is the brown bullhead *Ameiurus nebulosus* (IJC 1987), because this is a bottom-dwelling fish with a limited home range and is known to take up contaminants from food and sediments (Maccubbin et al. 1985, Baumann 1989, Baumann et al. 1991, 1996, Smith et al. 1994). Both liver (Harshbarger & Clark 1990, Baumann & Harshbarger 1995, Pinkney et al. 2001) and skin (Black 1983, Smith et al. 1989, Bowser et al. 1991, Poulet et al. 1994) neoplasms have been associated with exposure to polynuclear aromatic hydrocarbons (PAH) and other contaminants. Although the prevalence of skin neoplasia is often elevated in fish from chemically polluted, often industrial, sites (Sonstegard 1977, Hayes et al. 1990, Bowser et al. 1991), the association of epidermal papillomas and carcinomas with chemical exposure is less conclusive than with liver neoplasia (Poulet et al. 1994). There is also good evidence for the influence of PAH exposure on the development of liver neoplasm and other microscopic liver lesions in other fish species, including English sole *Parophrys vetulus* (Myers et al. 1990), mummichog *Fundulus heteroclitus* (Vogelbein et al. 1990) and winter flounder *Pseudopleuronectes americanus* (Gardner et al. 1989).

Numerous surveys have been undertaken to assess the prevalence of skin and liver tumors, as well as other contaminant-associated lesions at AOC. However, a major problem has been a lack of consistent criteria for evaluating histological changes in bullhead livers. For instance, in some studies there is no distinction between altered foci (preneoplastic) and neoplastic lesions, or inadequate descriptions of individual lesions (Black 1983, Pyron et al. 2001). Baumann et al. (2000) present a compilation of liver neoplasm prevalence at AOC by location and year. However, a comparison of the papers/reports from which these data were obtained demonstrates the lack of consistent criteria. For example, a prevalence of 7% was noted in the Ashtabula River in 1991 by Mueller & Mac (1994). In that study, neoplasia included only hepatocellular carcinoma (advanced liver neoplasms) or cholangiocarcinoma (biliary neoplasms), but not hepatocellular adenoma, cholangioma or altered foci (Mueller & Mac 1994). Conversely, the prevalence reported for the Black River in 1982 (60%) included carcinomas and adenomas (Baumann et al. 1990), while the 9% in the Detroit River in 1985 to 1987 included carcinomas, adenomas and altered foci (Maccubbin & Erasing 1991).

An RAP is required for each AOC. The intent is to provide a strategic guideline that outlines all phases of the remedial effort, from problem definition to active remediation, through to the recovery stage and, finally, delisting. A key component of these plans is the specification of realistic and attainable delisting targets and the collection of data necessary to demonstrate that the beneficial use is no longer impaired. For the 'fish tumors and other deformities' impairment, there are 2 primary issues that require resolution: (1) the standardization of the criteria being used to evaluate the impairment, and (2) the institution of realistic delisting targets. Criteria need to be established for both sampling and monitoring this impairment so that data from different years, reference sites and AOC can be compared.

Diagnostic criteria already exist for non-neoplastic and neoplastic liver lesions in some fish species such as English sole *Parophrys vetulus*, medaka *Oryzias latipes*, the common dab *Limanda limanda* and European flounder *Platichthys flesus* (Myers et al. 1987, Boorman et al. 1997, Feist et al. 2004). Even though liver lesions in a variety of fish species are very similar, it is useful to provide detailed descriptions and illustrations for species commonly used in laboratory studies and monitoring programs. Therefore, the purpose of this report was to address the need for diagnostic criteria for proliferative liver lesions in bullhead and to suggest a classification system for these lesions. Commonly observed non-neoplastic and neoplastic proliferative lesions are described and illustrated to provide guidelines to assist pathologists evaluating studies utilizing these fish as indicator or sentinel species, particularly in the Great Lakes.

MATERIALS AND METHODS

Material for this review was obtained from numerous field collections of brown bullheads, primarily from tributaries of Lake Erie but including other sites such as the Hudson River (New York), and ponds on Cape Cod (Massachusetts), where bullhead have been used as an indicator species for assessing environmental stress. These include liver samples from over 1500 brown bullhead collected from 1998 to the present, for which histologic slide collections are housed at the US Geological Survey's National Fish Health Research Laboratory, Kearneysville, West Virginia. These studies involved random samples of fish from specific sites, and the proliferative lesions observed microscopically were not often associated with grossly visible liver lesions. In addition, the National Cancer Institute's Registry of Tumors in Lower Animals, Sterling, Virginia, currently has 991 brown bullhead cases from numerous surveys dating from 1965 to 2001. Approxi-

mately 680 of these cases had previously been diagnosed with liver neoplasms and we reviewed 25 cases for specific lesions. Many of the cases in the Registry were associated with grossly observable lesions. Most of the above studies targeted fish 3 yr of age or older, with a total length of 250 mm or greater. Typically, tissue was fixed in 10% buffered formalin, embedded in paraffin, sectioned at 3 to 6 μm and stained with hematoxylin and eosin.

Slides were examined with a Nikon Eclipse E600 microscope with Plan Apochromatic lenses and a Spot RT Slider™ (Model 2.3.1) high resolution digital camera system (Diagnostic Instruments). Images were captured from the system using Spot™ (Version 3.3 for Windows®) capture software at 1600 \times 1200 dots per inch (dpi) resolution. Brightness/contrast adjustments were performed in Adobe Photoshop® 6.0 for Windows (Adobe Systems).

RESULTS

Normal hepatic histology of brown bullhead

As in most teleosts, the liver of brown bullhead is composed of hepatic tubules. The classic hexagonal architecture of the mammalian hepatic lobule is not evident in fish livers (Gingerich 1982, Vethaak & Wester 1996, Metcalfe 1998, Hinton et al. 2001). The appearance of teleost hepatocytes can vary greatly due to sex, maturity, diet, season, contaminant exposure and other factors (Hinton & Couch 1998, Rocha & Monteiro 1999). Normal hepatocytes may have little or no observable lipid or glycogen storage (vacuolization) (Fig. 1A) or may contain extensive amounts of these stored energy sources (Fig. 1B). Exocrine pancreatic tissue and macrophage aggregates are commonly present (Fig. 1C,D) and bile ducts may also be observed

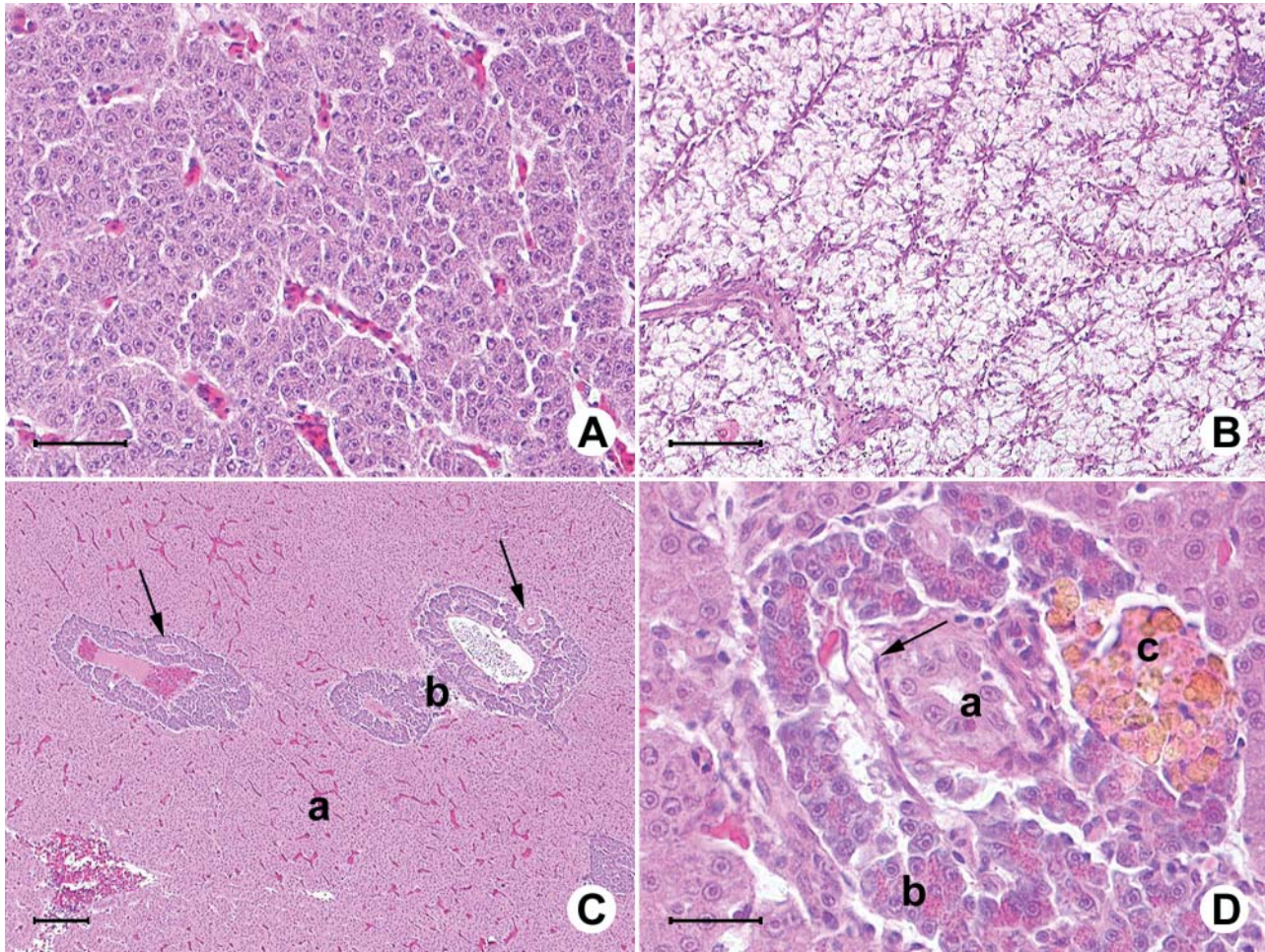


Fig. 1. *Ameiurus nebulosus*. Microscopic appearance of normal liver of brown bullhead. (A,B) Normal hepatocyte appearance can vary from (A) relatively unvacuolated to (B) significant vacuolization due to lipid and glycogen storage. Scale bars = 50 μm . (C) Within liver parenchyma (a), exocrine pancreatic tissue (b) often surrounds vessels and bile ducts (arrows). Scale bar = 100 μm . (D) Bile ducts (a) have a low columnar epithelium and are surrounded by a thin band of connective tissue (arrow); pancreatic tissue (b) and macrophage aggregates (c) are often observed in this area. Scale bar = 25 μm . Hematoxylin & eosin

within this tissue. In liver sections from bullheads collected at reference sites, bile ducts are often sparsely distributed in sections examined by light microscopy, although numerous bile preductules and ductules are located within the hepatic parenchyma (Hinton & Couch 1998). Small and intermediate bile ducts have columnar epithelium and the ducts are encircled by a thin band of connective tissue (Fig. 1D). Large bile ducts are surrounded by connective tissue and are prominent near the common bile duct.

Proliferative lesions in brown bullhead liver

As in higher vertebrates, proliferative lesions of either hepatocellular or biliary origin can be categorized as putative pre-neoplastic, non-neoplastic or neoplastic.

Putative pre-neoplastic hepatocellular lesions: foci of cellular alteration

Based on tinctorial characteristics of the hepatocyte cytoplasm, 4 categories of altered foci can be recognized in hematoxylin and eosin-stained sections. These include basophilic foci, eosinophilic foci, vacuolated cell foci and clear cell foci. Foci are generally distinct in terms of coloration, but the hepatic tubules are arranged in a relatively normal pattern, and they merge imperceptibly with the surrounding parenchyma with little to no compression. Features of cellular atypia (increased nuclear to cytoplasmic ratio, nuclear pleomorphism, nucleolar enlargement, or the presence of coarsely clumped chromatin) are not generally evident.

- Basophilic foci are round to irregular clusters of hepatocytes with increased basophilic staining compared to adjacent cells (Fig. 2A). There is no evidence of compression of adjacent unaffected hepatic tissues, little to no atypia of hepatocytes, and mitotic figures are generally not present. In some lesions, constituent cells may be smaller than adjacent hepatocytes (Fig. 2B).
- Eosinophilic foci are round to irregular areas of hepatocytes with increased eosinophilic staining compared to adjacent cells (Fig. 2C). There is little to no evidence of compression; however, the cells may be slightly enlarged with granular eosinophilic cytoplasm (Fig. 2D).
- Vacuolated cell foci are round to irregular, blend into the surrounding parenchyma without compression, and contain hepatocytes with clear cytoplasmic vacuoles of varying sizes (Fig. 2E). Nuclei are often eccentrically located within the cells (Fig. 2F). Because the cytoplasmic lipid is often present in a micro-

vesicular form, at low magnification the vacuolated foci may resemble clear cell foci.

Clear cell foci, as described in other fish species, with a 'ground glass' appearance of the cytoplasm, indicative of glycogen storage, and centrally located nuclei (Feist et al. 2004, Köhler 2004) were not observed in the bullhead livers examined. Occasionally individual cells within a vacuolated focus appeared to contain both lipid and glycogen and it is possible that bullhead foci may contain both storage products.

Neoplastic hepatocellular lesions

Hepatocellular adenoma

Adenomas are usually single discrete lesions with distinct borders (Fig. 3A). The cells often exhibit altered staining properties, either more eosinophilic or more basophilic than the non-neoplastic liver, and usually exhibit altered growth patterns (Fig. 3B). Macrophage aggregates, pancreatic tissue and other structures are often missing or sparse within adenomas (Fig. 3A–C). Adenomas are often well demarcated by compression of the adjacent parenchymal cells (Fig. 3C) and mitotic figures are rarely observed.

Hepatocellular carcinoma

Hepatocellular carcinomas are usually distinctly different from the surrounding liver tissue and have irregular borders due to invasion of neoplastic cells into the adjacent hepatic parenchyma (Fig. 3D). Carcinomas may be small or large lesions and may feature tumor giant cell formation. In some cases, foci of neoplastic cells are diffusely spread throughout the hepatic parenchyma (Fig. 3E). Cellular pleomorphism and nuclear atypia are key features, and there may be an increase in the number of mitotic figures and/or cells with multiple nucleoli (Fig. 3F).

Non-neoplastic biliary lesions: bile duct hyperplasia

Bile duct hyperplasia is characterized by an increased number of bile ducts (Fig. 4A) compared to normal liver (Fig. 4B). Hyperplastic bile ducts are often scattered and do not necessarily form discrete masses. The biliary epithelium is always well-differentiated; however, there may be moderate fibrosis associated with proliferating bile ducts (Fig. 4C), and the ducts tend to display substantial variation in size and shape. Occasionally the periductal fibrosis can be extensive (Fig. 4D).

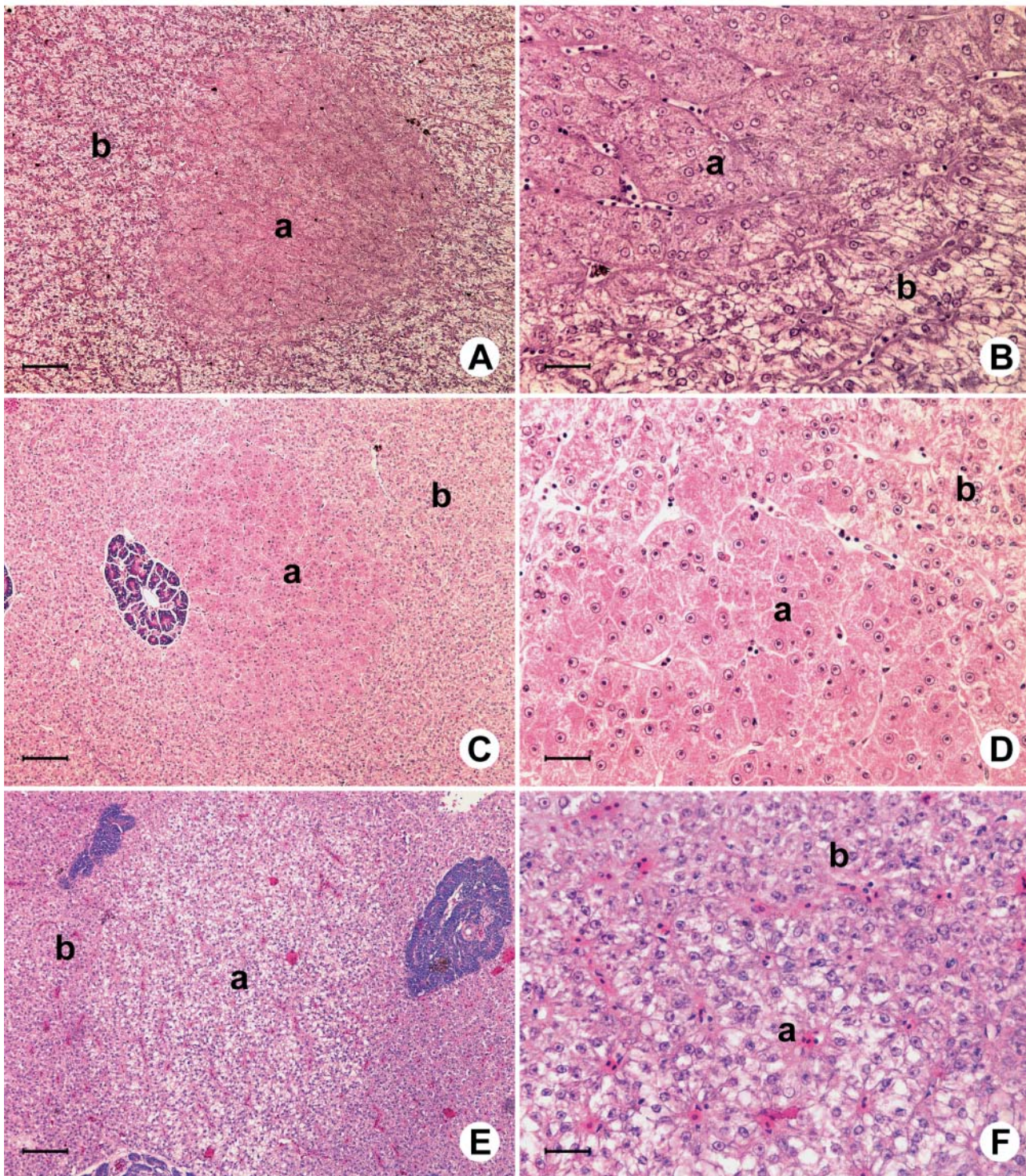


Fig. 2. *Ameiurus nebulosus*. Foci of cellular alterations in brown bullhead liver. (A) Basophilic focus (a) comprised of groups of hepatocytes with increased basophilic staining compared to surrounding hepatocytes (b). Scale bar = 100 µm. (B) Cells within basophilic focus (a) show little to no atypia and the border between these cells and surrounding hepatocytes (b) is distinct. Scale bar = 25 µm. (C) Eosinophilic focus (a) comprised of hepatocytes with increased eosinophilic staining compared to surrounding parenchyma (b). Scale bar = 100 µm. (D) Cells within an eosinophilic focus (a) may be slightly larger than surrounding cells (b) and have a granular cytoplasm. Scale bar = 25 µm. (E) Vacuolated focus is round to irregular and contains vacuolated hepatocytes (a) when compared to surrounding cells (b). Scale bar = 100 µm. (F) Cells within a vacuolated focus (a) contain vacuoles of varying size and nuclei may be peripherally located. Scale bar = 25 µm. Hematoxylin & eosin

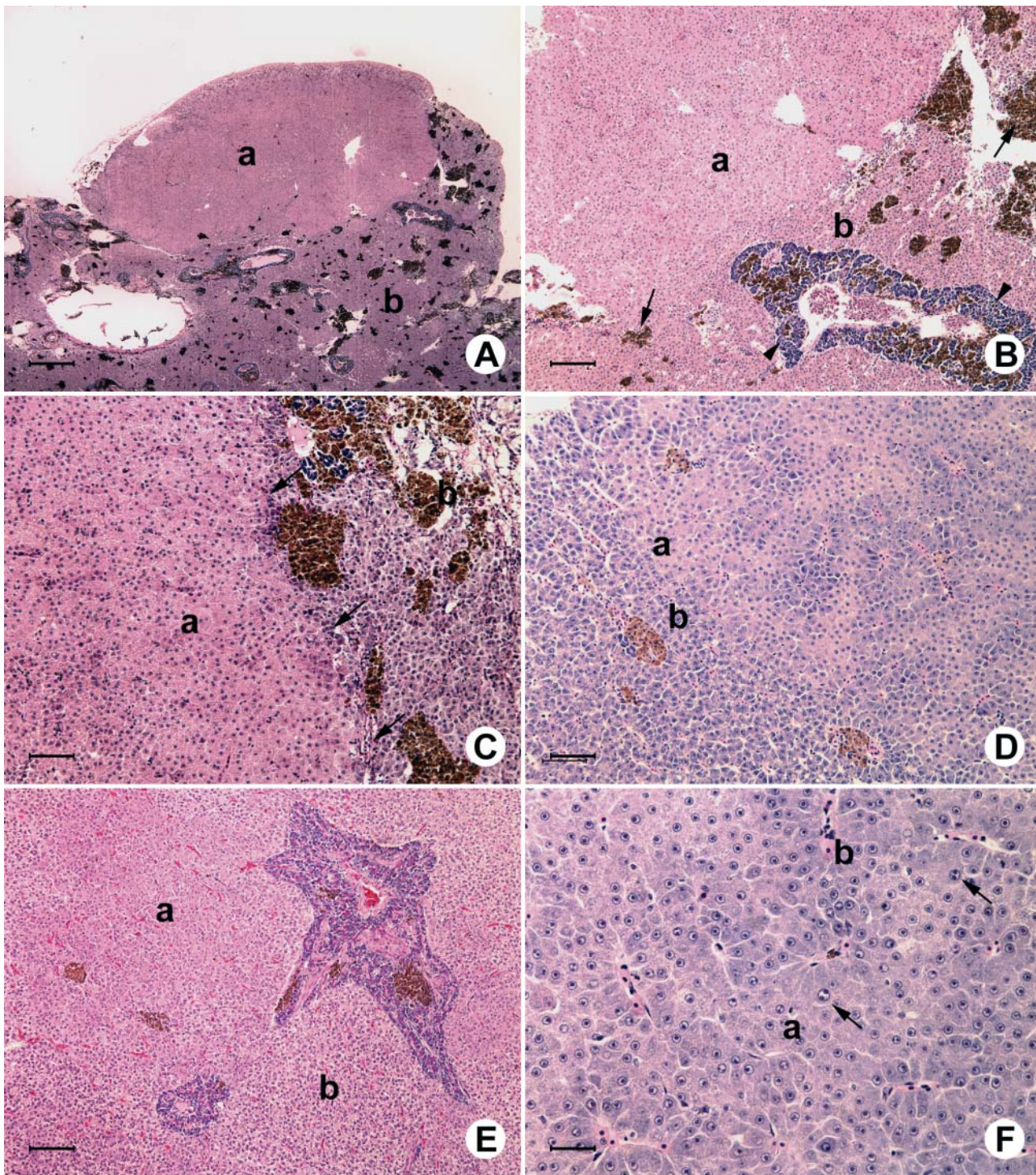


Fig. 3. *Ameiurus nebulosus*. Neoplastic hepatocellular lesions in brown bullhead liver. (A) Hepatocellular adenoma is a discrete nodule (a), well demarcated from normal tissue (b). Scale bar = 500 μ m. (B) Neoplastic cells within hepatocellular adenomas (a) often stain more eosinophilic than normal cells (b); pancreatic tissue (arrowheads) and macrophage aggregates (arrows) are generally absent or scarce within the adenoma. Scale bar = 100 μ m. (C) Cells within hepatocellular adenomas (a) usually exhibit altered growth patterns and are demarcated by compression of adjacent hepatocytes (arrows). Scale bar = 50 μ m. (D) Hepatocellular carcinoma showing irregular border with neoplastic cells (a) extending into and sometimes surrounding the normal hepatocytes (b). Scale bar = 50 μ m. (E) Hepatocellular carcinoma exhibiting a more diffuse appearance with multiple foci of neoplastic cells (a) interspersed with normal hepatocytes (b). Scale bar = 100 μ m. (F) Neoplastic cells within hepatocellular carcinoma are pleomorphic, exhibit nuclear atypia including multiple nucleoli (arrows). Scale bar = 25 μ m. Hematoxylin & eosin

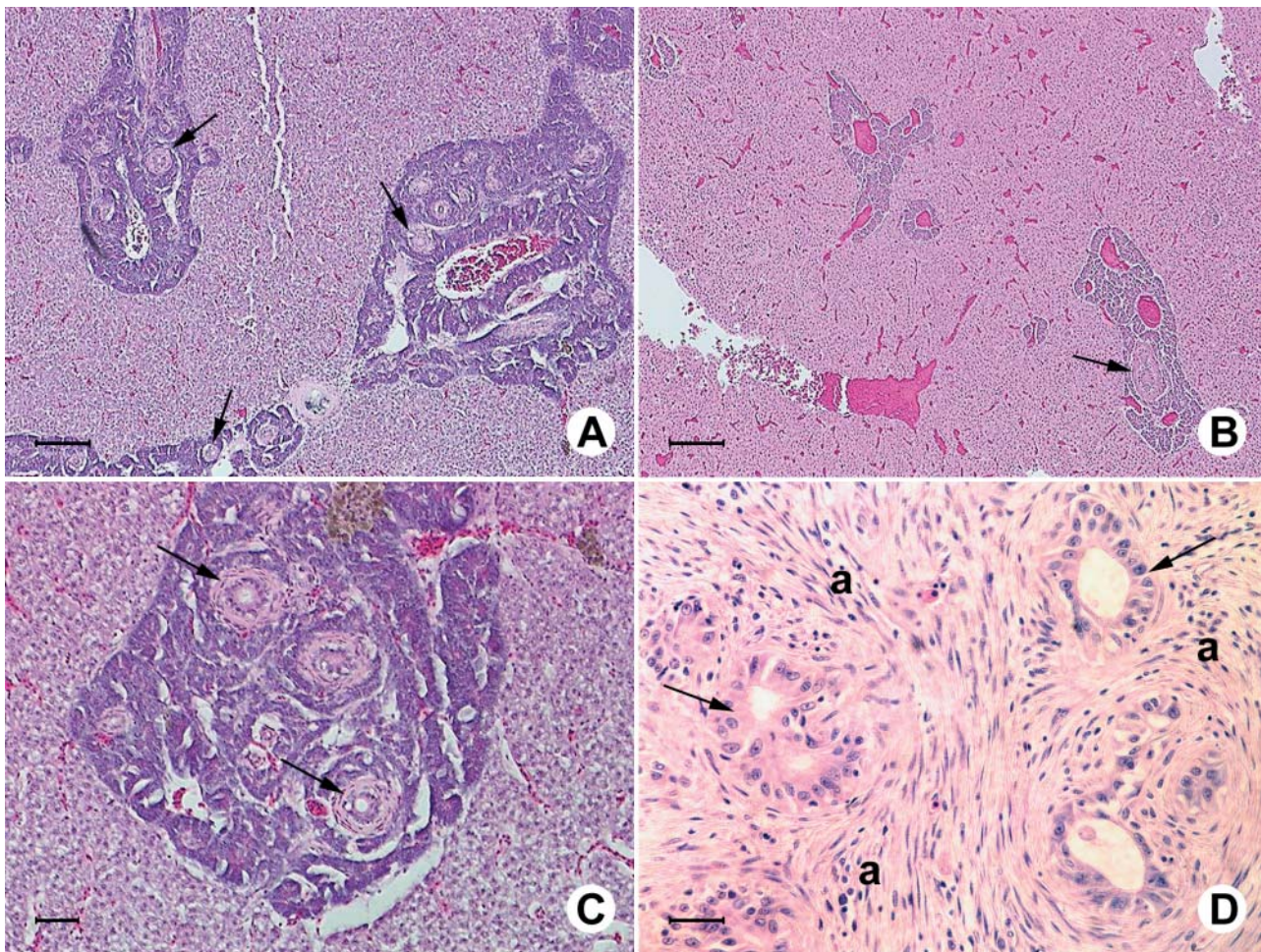


Fig. 4. *Ameiurus nebulosus*. Bile duct hyperplasia in brown bullhead liver. (A,B) Bile duct hyperplasia (A) in bullhead livers is characterized by an increased number of normal-appearing bile ducts (arrows) when compared to normal liver (B). Scale bars = 150 µm. (C) Hyperplastic bile ducts often have moderate fibrosis (arrows). Scale bar = 50 µm. (D) In some cases there is extensive fibrosis (a) around proliferating bile ducts (arrows). Scale bar = 50 µm. Hematoxylin & eosin

Neoplastic biliary lesions

Cholangioma

Cholangiomas are usually nodular, well-defined neoplasms (Fig. 5A) that consist of moderately well-defined bile ducts with little fibrous connective tissue. In cholangiomas, clusters of bile ducts form expansive masses with discrete borders. Some cholangiomas feature dilated, irregularly shaped bile ducts. The duct epithelium is usually 1 cell layer thick, there is mild to moderate cellular atypia, and mitotic figures are uncommon (Fig. 5B).

Cholangiocarcinoma

Cholangiocarcinomas are comprised of atypical proliferating bile ducts that are admixed with an abun-

dant, proliferating, fibrocellular stroma. These neoplasms display irregular, poorly defined borders where they often extend into the surrounding parenchyma (Fig. 5C,D). Proliferating bile ducts are usually irregularly shaped and the neoplastic epithelium is pleomorphic (Fig. 5E). The appearance of these tumors can vary from ductular structures within a moderately fibrous stroma (Fig. 5C) to poorly organized nests of biliary epithelial cells with numerous mitotic figures (Fig. 5F).

DISCUSSION

Laboratory studies on the pathogenesis of liver neoplasia in the bullhead are scarce. To our knowledge there is one documented experimental exposure in which 12 bullhead were fed a diet containing contaminated sediment extracts from the Black and Buffalo

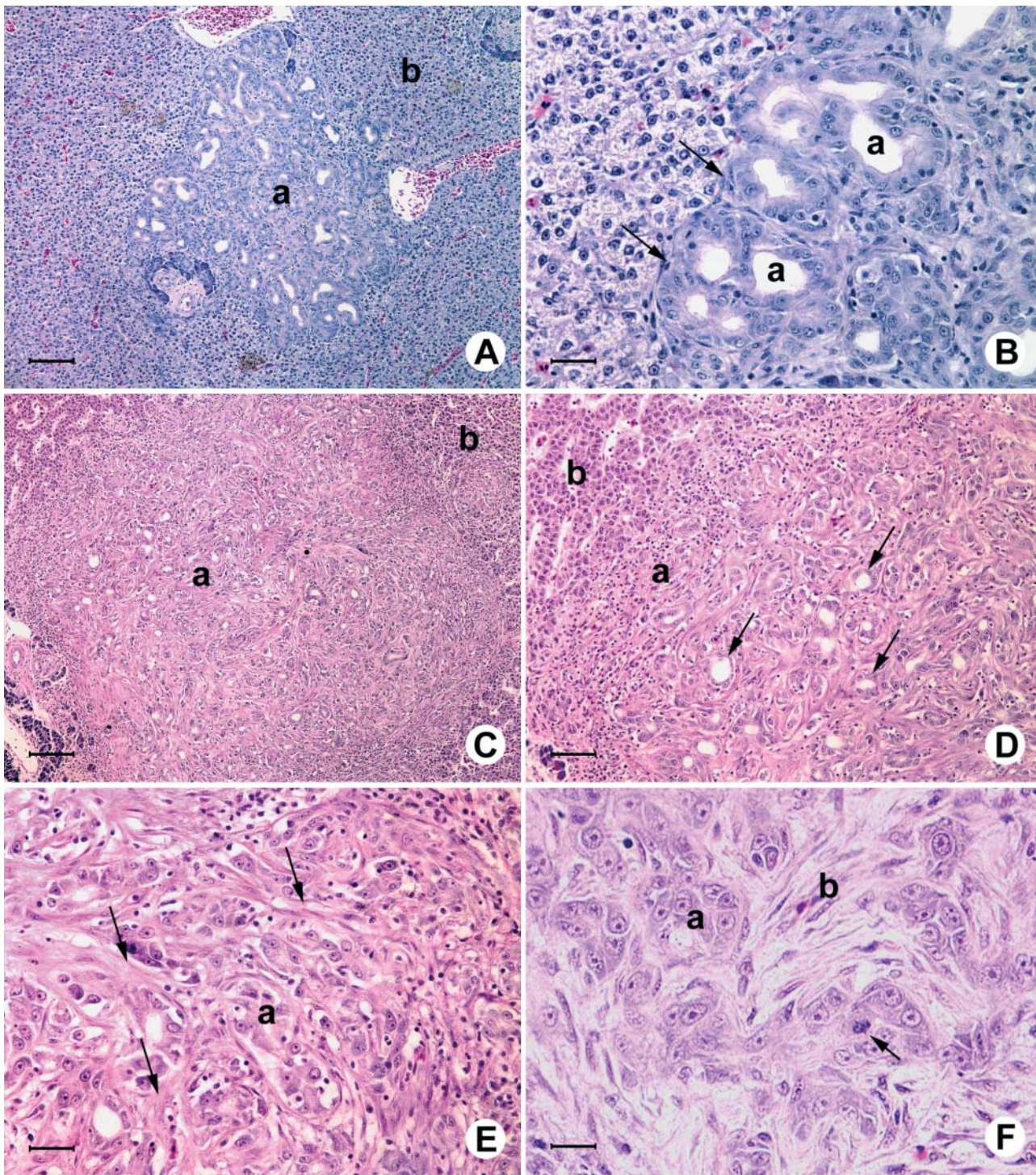


Fig. 5. *Ameiurus nebulosus*. Neoplastic biliary lesions in brown bullhead liver. (A) Cholangioma (a) is generally nodular and separated from normal hepatic tissue (b) by a well-defined border. Scale bar = 100 μ m. (B) Higher magnification of (A) showing well-defined border (arrows) and epithelium of bile ducts (a), usually 1 cell thick but may be dilated and irregularly shaped. Scale bar = 25 μ m. (C) Cholangiocarcinoma composed of atypical proliferating bile ducts (a) with irregular, poorly defined border extending into surrounding parenchyma (b). Scale bar = 100 μ m. (D) Higher magnification of (C) illustrating neoplastic bile ducts extending into normal hepatic parenchyma (b), eliciting an inflammatory reaction (a) at the border; normal-appearing ducts can still be observed in some areas (arrows). Scale bar = 50 μ m. (E) Cholangiocarcinoma composed of irregularly shaped ducts (a) within a fibrocellular stroma (arrows). Scale bar = 25 μ m. (F) In some cases, cholangiocarcinomas are composed of poorly organized nests of biliary epithelial cells (a) within a fibrocellular stroma (b); mitotic figure (arrow) can be observed. Scale bar = 25 μ m. Hematoxylin & eosin

rivers (Black et al. 1985). In that experiment, 6 of the fish on the experimental diet were sacrificed after 4 mo and 6 were terminated after 7 mo. Lesions identified in the fish sacrificed at 4 mo included a basophilic focus, a small focus of bile duct proliferation, and a larger lesion consisting of mixed fibrosis and duct-like formations of hepatocytes. At 7 mo, findings included numerous clear cell and eosinophilic foci, bile duct proliferation and fibrosis, and a cholangioma. In a second study, brown bullhead were injected with a single intraperitoneal dose of 0, 5, 25, or 125 mg kg⁻¹ benzo[a]pyrene and evaluated over 18 mo (Grady et al. 1992); one focus of biliary hyperplasia and fibrosis in a bullhead exposed to 25 mg kg⁻¹ and basophilic foci at all exposure levels were observed at Day 540. None of these lesions were observed in control fish. Unfortunately, none of the lesions in either study were described in detail or illustrated, nor was there any mention of control fish in the first study. Hence, further, more definitive studies are necessary to determine whether non-neoplastic proliferative lesions should be regarded as preneoplastic precursors of hepatocellular or biliary neoplasms in bullhead.

Foci of cellular alteration (basophilic, eosinophilic, vacuolated and clear cell) have been reported in neoplastic epizootics as well as carcinogenesis studies. There is evidence for the progression of basophilic foci to hepatocellular carcinoma in rainbow trout *Oncorhynchus mykiss* (Hendricks et al. 1984), medaka *Oryzias latipes*, guppies *Poecilia reticulata* (Hawkins et al. 1990), mosquitofish *Gambusia affinis* (Law et al. 1994), and sheepshead minnow *Cyprinodon variegatus* (Hinton et al. 1988), whereas there is no clear indication that other altered foci progress to neoplasia (Bunton 1996). However, several types of foci (vacuolated cell, eosinophilic and basophilic) that were observed in rivulus *Rivulus marmoratus* exposed to diethylnitrosamine were considered preneoplastic (Grizzle & Thiyagarajah 1988). Altered foci are also considered preneoplastic in wild English sole exposed to PAHs in Puget Sound (Myers et al. 1987, 1991), winter flounder from contaminated sites in Boston Harbor (Murchelano & Wolke 1991), flounder from Dutch coastal areas (Vethaak & Wester 1996), and British estuaries impacted by contaminants, including PAHs (Stentiford et al. 2003). Indeed, there is substantial evidence that increased prevalences of altered foci are associated with contaminant exposure, and should therefore be considered potential indications of such exposure and putatively pre-neoplastic.

Cholangiolar neoplasia has also been induced in numerous species through experimental contaminant exposures (Hendricks et al. 1984, Parland & Baumann 1985, Couch & Courtney 1987, Grizzle & Thiyagarajah

1988, Hawkins et al. 1988, Law et al. 1994, Boorman et al. 1997). As with hepatic lesions, it has not been experimentally demonstrated which, if any, non-neoplastic cholangiolar changes may be considered preneoplastic in the bullhead. Proliferation of bile ductular epithelial cells has been observed during experimental chemical exposures in a number of species (Hendricks et al. 1984, Parland & Baumann 1985, Couch & Courtney 1987), and in such fishes the cells may be considered preneoplastic (Bunton 1996). Bile duct proliferation and fibrosis have also been reported in association with hepatic neoplasia in other fish species at a number of contaminated sites and are considered toxicopathic, and in some cases preneoplastic (May et al. 1987, Moore et al. 1989, Vogelbein et al. 1990, Baumann et al. 1991, Stehr et al. 2003). However, in bullheads, a myxosporidian parasite can commonly be observed within the lumen of bile ducts (Fig. 6A,B) and it is possible that bile duct proliferation and fibrosis may be related to the presence of this or other parasites. Similarly, a proliferative inflammatory response and giant cell formation has been observed in association with helminth parasites in the parenchyma of bullheads (Fig. 6C,D) and should not be confused with toxicopathic lesions.

In addition to the need for consistent diagnostic criteria, there is a need to establish consistent sampling procedures. A quality control issue for detection of neoplastic and other proliferative liver lesions concerns the number of histologic sections or levels in a liver that are necessary to accurately estimate the presence and extent in an individual liver. Stine et al. (2004) evaluated 6 livers from adult mummichog (a much smaller fish species than bullhead), 5 of which had grossly visible nodules. The authors discovered that: cancerous and precancerous lesions are not homogeneously distributed; the presence of one lesion type may influence the extent and distribution of other lesions; multiple sections are needed (an average of 6 sections for 50 % of lesions to be identified or 7 sections for 95 % of lesion estimates to be included); and tissues sectioned in different planes and observations from different sections will generate different lesion observations and conclusions. In our studies, very few of the proliferative lesions diagnosed in bullhead were grossly apparent. Hence, it must be recognized that for large fish species such as adult brown bullhead more than 10 sections at various locations throughout the liver may be necessary to adequately estimate lesion prevalence.

One problem with the IJC 'fish tumors and other deformities' beneficial use impairment is that the phrase 'neoplastic or preneoplastic liver tumors' was never clearly defined. Certainly, hepatocellular adenoma, hepatocellular carcinoma, cholangioma and cholan-

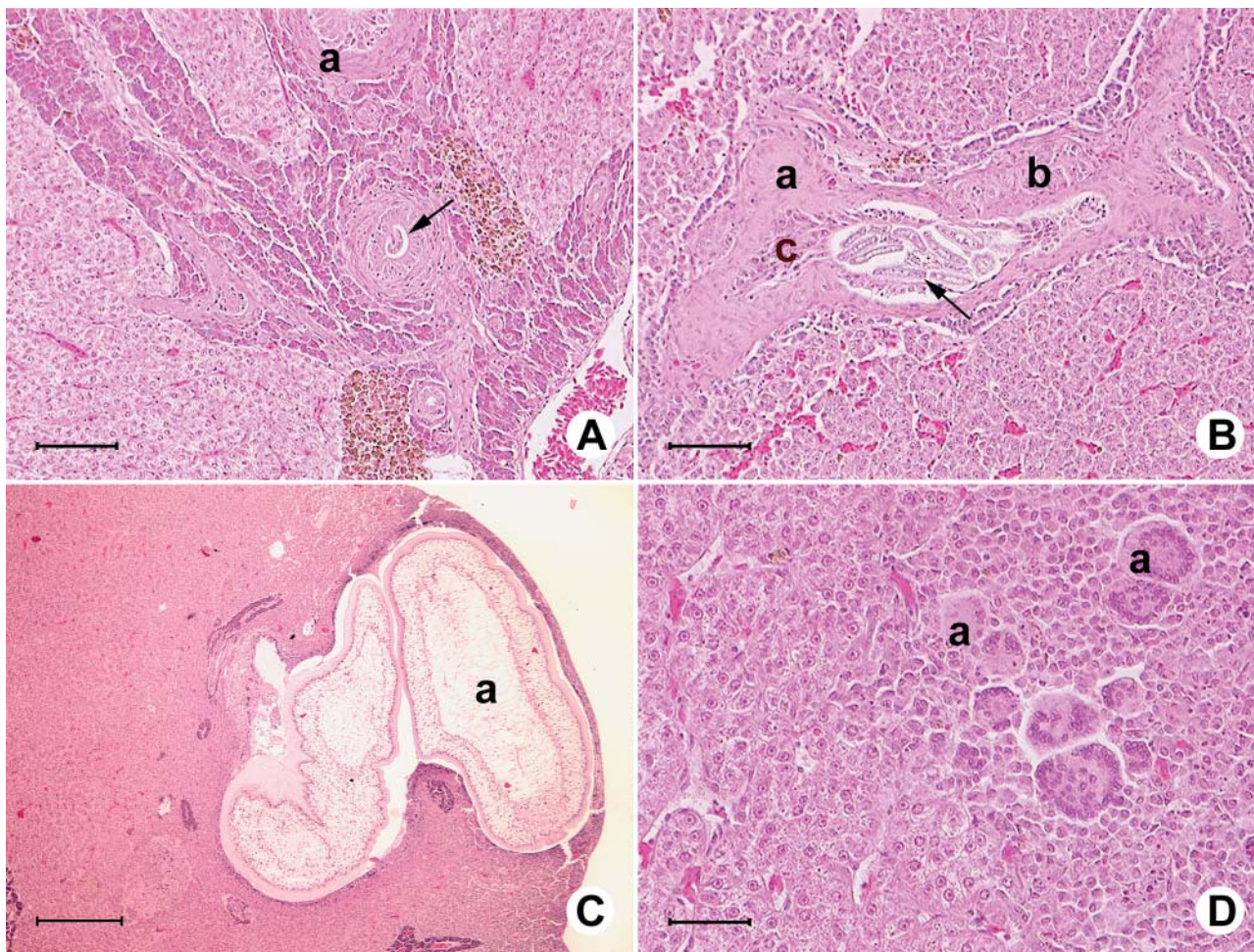


Fig. 6. *Ameiurus nebulosus*. Parasitic lesions in brown bullhead livers. (A,B) Fibrosis (a), ductule proliferation (b) and hyperplasia of ductule epithelium (c) can be observed around bile ducts that contain myxosporidian parasites (arrows). Scale bars = 100 µm. (C) Raised nodule in liver parenchyma caused by helminth parasite (a). Scale bar = 250 µm. (D) Inflammatory response to helminth parasites may include giant cell (a) formation. Scale bar = 50 µm. Hematoxylin & eosin

giocarcinoma are neoplastic lesions that should be reported as such. Foci of cellular alteration, although not definitely shown to be preneoplastic in bullheads, should be viewed as putatively pre-neoplastic and should be documented in monitoring efforts. Bile duct proliferation and fibrosis may be related to contaminant exposure in some instances, but these may also be a result of parasite infection. Such lesions should not be considered preneoplastic, but should be documented and considered as potential toxicopathologic indicators.

Of necessity, survey studies that are used to assess the progress of AOC remediation efforts must be conducted at multiple time points that often span several years. Thus, it is inevitable that individual studies will be evaluated histologically by different pathologists, each of whom may have different training experiences and levels of expertise. Because of these factors, and

the semi-subjective nature of histopathological evaluations, the consistency of results across studies may be somewhat less than desirable, and hence far from ideal for regulatory purposes. Thus, it would be desirable for each individual study to be peer-reviewed by a second pathologist who, like the initial pathologist, is familiar with the specific diagnostic criteria as described above. In addition, prior to the assimilation of study data into the regulatory decision-making process, we recommend that the collection of relevant studies be reviewed for diagnostic consistency and accuracy by a panel of fish pathology experts in a 'Pathology Working Group' format (Boorman et al. 1997). Slide collections such as those housed at the Registry for Tumors of Lower Animals are invaluable for comparative purposes, for retrospective analyses and development of diagnostic criteria for numerous lesions and fish species.

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