Neoplastic and Nonneoplastic Hepatic Changes in Lake Whitefish (*Coregonus clupeaformis*) from the St. Lawrence River, Quebec, Canada

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As part of a survey of fish diseases, lake whitefish (*Coregonus clupeaformis*) were collected in fall 1995 from the St. Lawrence River 15 km upstream of Quebec City, Quebec, Canada, to assess the prevalence of liver lesions. A total of 141 fish were captured and necropsied, and three standard sections of liver were taken for histological examination. Prevalences of altered hepatocyte foci, hepatocellular carcinoma, cholangioma, and cholangiocarcinoma were 0.7%, 2.1%, 0.7%, and 2.1%, respectively. Thus, the overall prevalence of liver neoplasia was 4.9% (7/141). Hepatic tumors were only observed in fish 7 years old or older. Fish age was significantly and positively correlated with the index assessing the number and size of macrophage aggregates ($p<0.001$; $r_s = 0.16$). Hepatocyte vacuolation, anisokaryosis, lymphocytic infiltration, and bile duct hyperplasia were also observed but were not related to the age, length, sex, or condition factor of the fish. These results represent the first report on a series of hepatic tumors in a wild salmonid species. *Key words*: cancer, environmental carcinogenesis, fish, liver, pathology. *Environ Health Perspect* 106:179–183 (1998). [Online 26 February 1998] *http://ehpnet1.niehs.nih.gov/docs/1998/106p179-183mikaelian/abstract.html*

The occurrence of hepatic neoplastic and certain associated nonneoplastic lesions over 20 fish species has been attributed to the presence of chemical carcinogens in aquatic ecosystems (*1–8*). Consequently, monitoring hepatic lesions in target fish species may provide an indicator of pollution (*9–12*).

In 1994 and 1995, a general assessment of the health of the St. Lawrence River was sponsored by Environment Canada, and fish diseases were monitored as part of this program. In 1994, 16 of 197 lake whitefish (*Coregonus clupeaformis*) from the St. Lawrence River were selected for necropsy on the basis of external abnormalities during a survey at Saint-Nicolas, Quebec (*13*). Two of these 16 fish were diagnosed with cholangiocarcinomas [Registry of Tumors in Lower Animals (RTLA), #6185 and #6186], but no liver mass was found upon macroscopic examination of 1,291 randomly selected fish belonging to 15 species, including 297 channel catfish (*Ictalurus punctatus*), 200 longnose suckers (*Catostomus catostomus*), and 184 white suckers (*Catostomus commersoni*). The present study was undertaken in 1995 to determine more precisely the prevalence of liver neoplasms and other hepatic lesions in lake whitefish from the St. Lawrence River and to examine the effects of fish age and size on the occurrence of these pathological conditions.

Materials and Methods

Lake whitefish were collected using fixed fishing gear installed at Saint-Nicolas (46°43' N, 71°19' W), Quebec, Canada, 15 km upstream of Quebec City, from 30 August 1995 to 30 October 1995. All lake whitefish caught during this period were kept for examination.

Upon collection, fish were measured for total length to the nearest millimeter and weighed to the nearest gram. Fish were humanely euthanized with an overdose of tricaine methane sulfonate (MS 222), and a complete postmortem examination was performed. The weight of liver, gonads, and gastric content and the eviscerated weight were recorded.

Samples of liver and of any internal lesion were collected from every fish. Three standard sections of liver were preserved in 10% buffered formalin for histological analyses. Samples were routinely embedded in paraffin wax, sectioned at 5 µm, and stained with hematoxylin-phloxin-saffron. Scales were removed from the ventrolateral side of the body and fish age was determined by scale reading with a stereomicroscope. Due to the difficulty of precisely determining the age of older fish with scale reading, all fish 9 years old or older were grouped as 9+ year fish.

All sections were examined by the same pathologist; lesions were further examined by a second pathologist, and tumors were confirmed by the RTLA. Foci of altered hepatocytes and liver neoplasms were classified according to criteria outlined by Boorman et al. (*14*). Other hepatic lesions were classified and graded as described below.

Hepatocyte vacuolation was graded 0 (no vacuolation), 1 (<5% hepatocytes were affected), 2 (5–40% were affected), and 3 (>40% were affected). The macrophage aggregates (MAs) were scored 0 (no MAs), 1 (presence of a few MAs scattered within the parenchyma), 2 (presence of numerous MAs), and 3 (more than 20% of the section consisted of MAs). Lymphocytic infiltration was graded 0 (no lymphocytes), 1 (a few lymphocytes and/or plasma cells around a few bile tracts), 2 (some bile tracts surrounded by 1–3 layers of lymphocytes and/or plasma cells), and 3 (some bile tracts surrounded by >3 layers of lymphocytes and/or plasma cells). Bile duct hyperplasia was graded 0 (no increase), 1 (slight increase in the number of bile ducts within a few randomly distributed portobiliary tracts), 2 (several markedly convoluted bile ducts within most portobiliary tracts or randomly scattered in the hepatic parenchyma; occasional loss of polarity or luminal infolding of the epithelium), and 3 (numerous markedly convoluted bile ducts in the hepatic parenchyma and/or in portobiliary tracts; prominent loss of polarity and anisokaryosis). Hepatocyte anisokaryosis was graded 0 (absence) and 1 (presence). Individual fish were scored according to the highest grade diagnosed on three microscopic sections.

Fish condition factor (CF), used as an overall physiological index, was calculated as follows:

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We are grateful to Fernand Gingras for his commitment to the collection of specimens; to Denis Labonté, Sylvie Legendre, and David Routier for field work; and to Jacinthe Cardin, Jules Deslandes, Lyne Pépin, and Bibiane Pépin-Faille for skillful laboratory assistance. Funded by St. Lawrence Vision 2000, Environment Canada, Montreal, Quebec; the Canadian Cooperative Wildlife Health Centre, Saskatoon, Saskatchewan; and the Centre Québécois sur la Santé des Animaux Sauvages, Saint-Hyacinthe, Quebec, Canada.

Received 5 August 1997; accepted 5 December 1997.

$$CF = \frac{100 \times (\text{total weight} - \text{gonad weight})}{(\text{length})^3}$$

where weight is expressed in grams and length in centimeters.

The difference between tumor prevalence in categories of length, age, and sex was statistically evaluated by a χ^2 test. CF of fish with and without hepatic lesions was compared using a Wilcoxon-rank-sum test. The relationship between the prevalence of each kind of lesion and fish age and CF was tested using Spearman rank correlation.

Results

The lake whitefish liver is composed of two large lobes suspended over the digestive tract from a ligament. These features facilitate easy removal and examination of the liver.

The MAs score increased with age (Fig. 1; Spearman correlation, $r_s = 0.16$; $p<0.001$; $n = 141$). Various degrees of hepatocyte vacuolation, anisokaryosis, nuclear pleomorphism, bile duct hyperplasia, lymphocytic proliferation, and foci of coagulative necrosis were found, irrespective of the length, sex, and CF of the fish or the presence of neoplastic lesions (Table 1).

Eosinophilic foci of altered hepatocytes were found in one fish (RTLA # 6354; Table 2). They consisted of two round and sharply demarcated areas that were 300 µm and 1 mm in diameter and composed of slightly enlarged hepatocytes with an abundant eosinophilic and finely granular cytoplasm (Fig. 2).

A total of seven fish were found with hepatic tumors, which yields an overall prevalence of 4.9% (7/141). Fish with hepatic tumors were 7 years of age and older and were longer than 450 mm (Table 2). The prevalence of hepatic tumors is 8.4% (7/83) for fish aged 7 years or older and 11.4% (4/35) for fish aged 9 years or older.

Hepatocellular carcinomas were found in three females. They consisted of poorly demarcated, translucent (RTLA #6264 and #6265) or pale brown (RTLA #6265), fleshy nodules located within the hepatic parenchyma. Microscopically, these tumors were nodular, single to multiple, poorly demarcated, mildly compressive, and unencapsulated (Fig. 3). They were composed of two- to three-cell thick irregularly convoluted trabeculae that were generally perpendicular to the margins of the mass. Neoplastic cells were about 50% larger than normal hepatocytes and their cytoplasm was slightly more basophilic. Their nuclei, 50% larger than normal, showed moderate hypochromasia and minimal anisokaryosis and each had a single large nucleolus that was eccentrically located and amphophilic. The nucleus/cytoplasm ratio was normal, and there were few mitoses (0–2/high power field). MAs and bile ducts were not present in tumors. In one specimen (RTLA #6260), neoplastic trabeculae were focally separated by dilated sinusoids measuring up to 100 µm in width.

A cholangioma was found histologically in one fish (Fig. 4). It consisted of a single, well-delimited and unencapsulated cluster of well-differentiated to slightly irregular bile duct-like structures that were separated by a small amount of fibrovascular stroma.

Cholangiocarcinomas were found macroscopically in one fish (RTLA #6258) and histologically in two additional fish (RTLA #6261 and #6266; Table 2). Macroscopically visualized tumors consisted of well-demarcated white solid nodules located within the parenchyma. Microscopically, these tumors were unencapsulated nodules, 0.3–3 mm in diameter, that were single or multiple, well-defined, locally infiltrative, and consisting of irregularly shaped bile duct-like structures separated by variable amounts of well-vascularized stroma (Fig. 5). Bile duct-like structures located at the center of the tumors were often dilated, some being cystic, with occasional infolding of the flattened epithelium. Peripheral bile duct-like structures were lined by a cuboidal epithelium, and their smaller diameter was less variable than that of the peripheral structures. Tumor cells had indistinct borders; variable amounts of pale acidophilic, finely granular cytoplasm; and an elongated, often monstrous, vesicular nucleus with a small basophilic nucleolus. A few neoplastic cells were binucleated. Anisokaryosis was more apparent at the periphery of tumors, and the nucleus/cytoplasm ratio was elevated (1/2 to 2/1). Mitoses were rare (less than 1/×400 power field).

Changes in other organs with gross lesions were not neoplastic and are not reported here.

Discussion

The lake whitefish population in the St. Lawrence River, 15 km upstream from Quebec City, seems to be affected by a high prevalence of hepatic neoplasia. Prior to this study and the previous report from our group (*13*), a single hepatic neoplasm (a cholangiocarcinoma) had been diagnosed in a fish from the genus *Coregonus* (lake herring, *Coregonus artedi*; Essex County, NY; RTLA #1426). To our knowledge, the present report is the first description of a series of hepatic neoplasia in a wild population of salmonid fish, although the domesticated

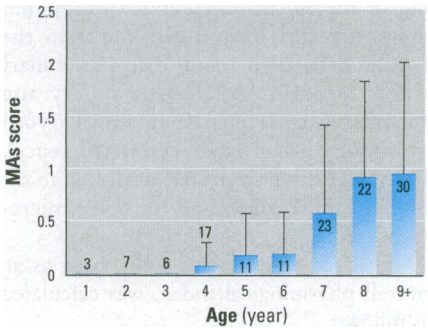

Figure 1. Mean index (± 1 standard deviation) of the number and size of macrophage aggregates (MAs) as a function of fish age. The numbers above or in the bars represent the sample size for each age class.

Table 1. Severity of nonneoplastic hepatic lesions in lake whitefish (*Coregonus clupeaformis*) caught in 1995 in the St. Lawrence River at Saint-Nicolas, Québec, Canada ($n = 141$)

	Histopathologic index			
Lesion	0	1	2	3
Hepatocyte vacuolation	77	22	28	14
Macrophage aggregates	96	20	21	4
Lymphocytic infiltration	40	93	8	0
Bile duct hyperplasia	69	66	5	1
Foci of coagulative necrosis	137	4		
Nuclear pleomorphism	139	2		

Table 2. Preneoplastic and neoplastic hepatic lesions in lake whitefish (*Coregonus clupeaformis*) caught in 1995 in the St. Lawrence river at Saint-Nicolas, Québec, Canada

	RTLA number	Length (mm)	Age	Sex	Number of lesions	Lesion diameter (mm)
Foci of altered hepatocytes	6354	489	6	M	2	1
Hepatocellular carcinoma	6264	523	9	F	±15	2–6
	6265	521	9+	F	3	1–3
	6260	547	9+	F	1	14
Cholangioma	6262	478	7	M	1	0.3
Cholangiocarcinoma	6258	532	9+	F	2	0.4–2
	6261	506	8	M	2	1
	6266	458	7	M	1	3
Fish without neoplasia	–	–	–	69 F	–	–
				60 M	–	–
				4 U	–	–

Abbreviations: RTLA, Registry of Tumors in Lower Animals; F, female; M, male; U, unknown.

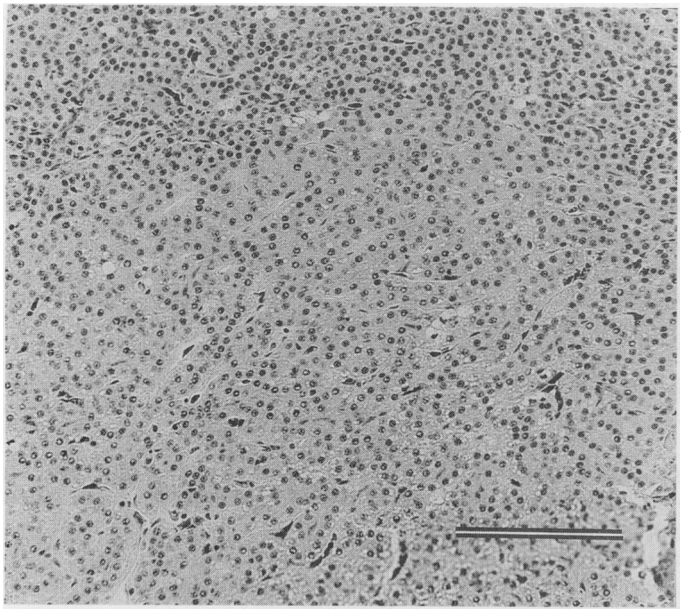

Figure 2. Eosinophilic foci of altered hepatocytes in lake whitefish (*Coregonus clupeaformis*). Altered hepatocytes form anastomosing trabeculae (two cells thick). Their cytoplasm is slightly more acidophilic and more abundant than normal hepatocytes. Bar = 200 µm; hematoxylin-phloxin-saffron.

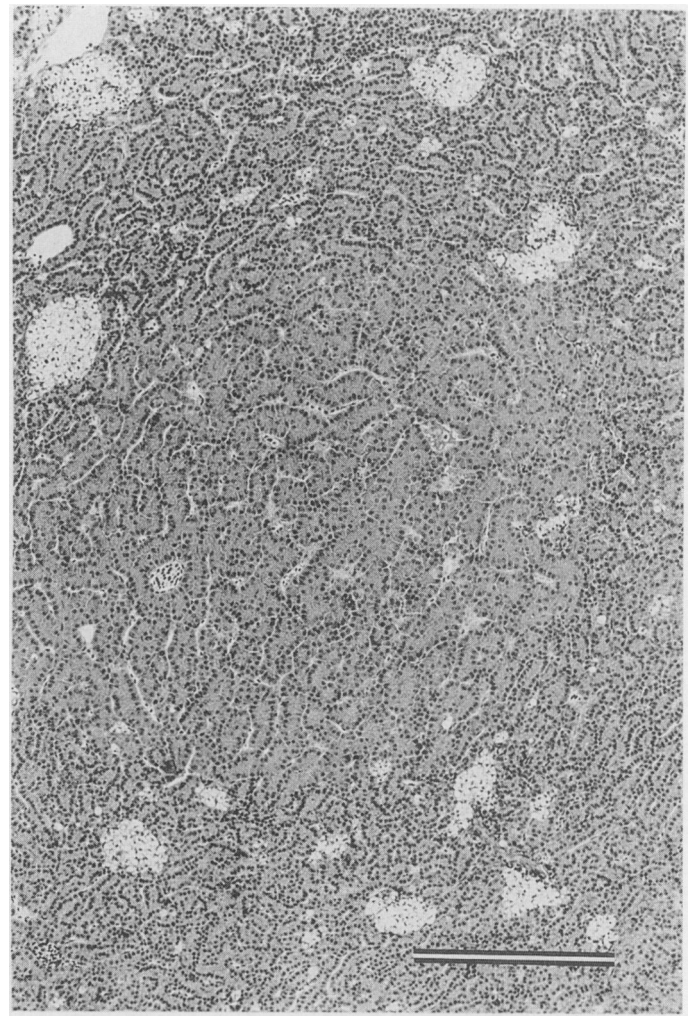

Figure 3. Hepatocellular carcinoma in lake whitefish (*Coregonus clupeaformis*). The tumor is composed of cords 2–4 cells thick. The tumor slightly compresses the adjacent parenchyma and is devoided of melanomacrophage aggregates and bile duct tracts. Bar = 300 µm; hematoxylin-phloxin-saffron.

rainbow trout is susceptible to a variety of carcinogenic compounds that cause hepatocellular carcinomas (*15–18*).

In humans and in other mammals, common causes of hepatic neoplasia are chemical carcinogens and viruses (*19–21*). *Helicobacter* infections (*22,23*) and parasitic liver flukes (*24,25*) have been associated with liver cancer. In feral fish, high prevalences of liver neoplasms have generally been attributed to anthropogenic (*2,3,6,7, 9,26–28*) and occasionally to natural (*29*) chemical contamination of the environment. Hepatic neoplasms were not detected in lake whitefish exposed to bleached kraft mill effluent subject to primary treatment (*30*) or in those inhabiting unpolluted Ontarian (*2,30*) and Quebec lakes (*31*). However, these studies are not comparable because histology was not done (*2,31*) or was done on a limited number of fish (*30*).

All previous series of hepatic neoplasms reported in wild fish occurred in bottom-feeding nonsalmonid species (*5,27*). Because benthic invertebrates are known to concentrate contaminants including polychlorinated biphenyls and powerful carcinogens such

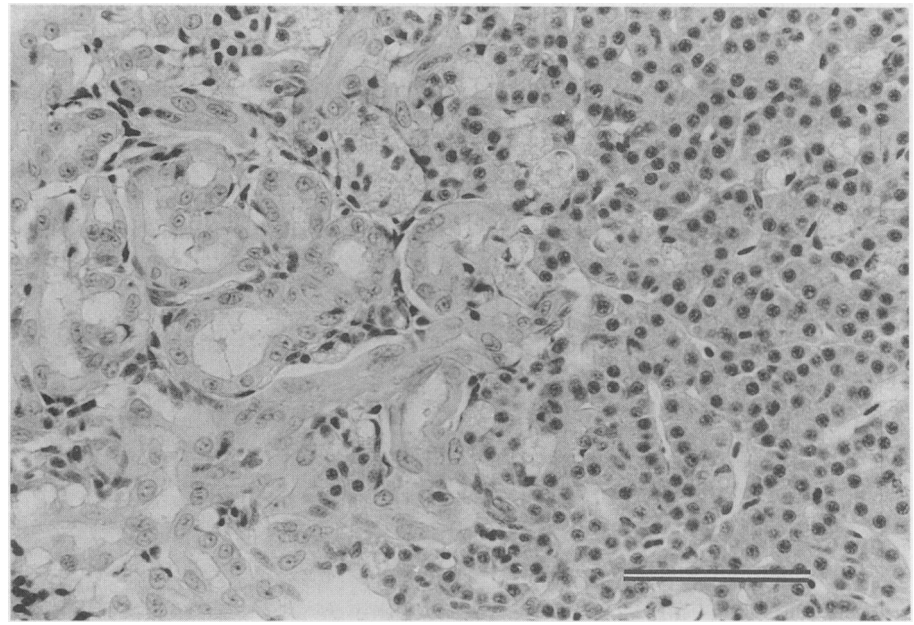

Figure 4. Cholangioma in lake whitefish (*Coregonus clupeaformis*). The tumor forms a small unencapsulated cluster of well-differentiated bile duct-like structures. Bar = 75 µm; hematoxylin-phloxin-saffron.

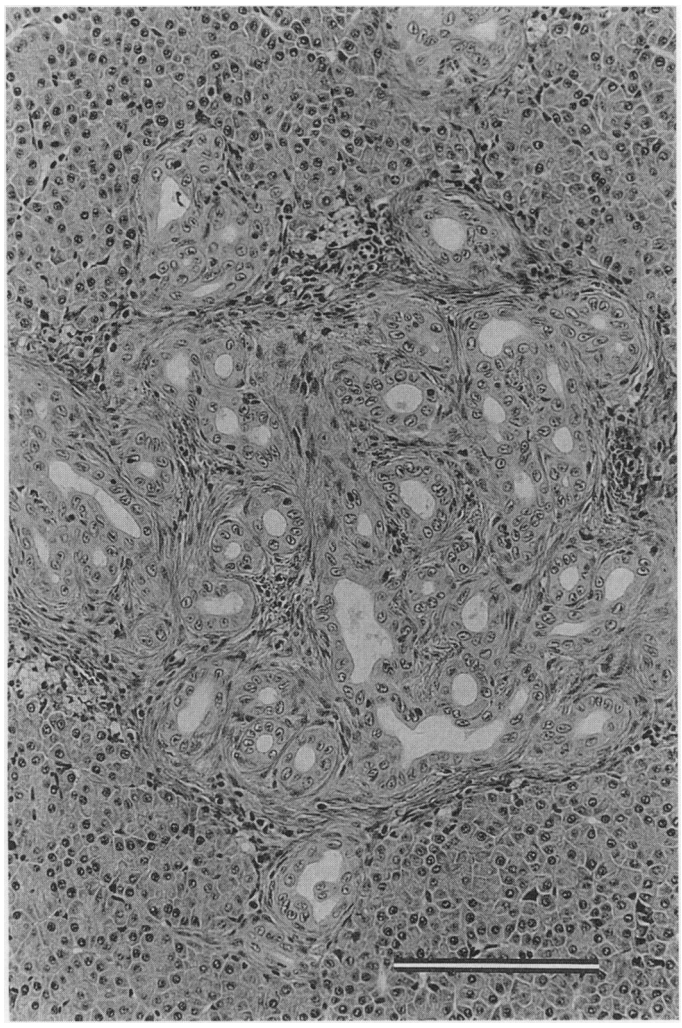

Figure 5. Cholangiocarcinoma in lake whitefish (*Coregonus clupeaformis*). The tumor is composed of closely packed, well-differentiated bile duct-like structures that infiltrate the adjacent hepatic parenchyma. Bar = 150 µm; hematoxylin-phloxin-saffron.

We found hepatocyte vacuolation and anisokaryosis. These changes have also been associated with exposure to contaminants in fish (*12,42*). However, these nonneoplastic changes were not associated with the presence of tumors, sex, age, or CF, and their significance remains undetermined.

Lake whitefish populations inhabiting large rivers in northern Quebec are anadromous (*43*). In the St. Lawrence River, reports by local fishermen and our observations (unpublished data) are consistent with an anadromous reproductive pattern. Consequently, the occurrence of hepatic neoplasms in lake whitefish, as opposed to their absence in the other fish species that we examined from the same area, may reflect the exposure of lake whitefish to different chemical compounds. It may also reflect differences in the ability to generate carcinogenic metabolites from xenobiotic compounds such as PAHs (*44*). Further studies assessing the relationship of contaminant levels in lake whitefish tissues to their diet are required to support the hypothetical link between contaminants and neoplasia in this species and to document the spatial movement and distribution of lake whitefish in the St. Lawrence River.

as polycyclic aromatic hydrocarbons (PAHs) (*32–35*), fish feeding on benthos are exposed to higher doses of carcinogenic contaminants. The gastric content of our lake whitefish consisted of gastropods admixed with varying numbers of amphipods (*Gammarus* spp.) and small amounts of sediments (de Lafontaine, unpublished observation), which indicates that lake whitefish feed on benthic organisms (*30,36*). Such a diet may play an etiological role in this series of tumors.

The MA score was statistically associated with age. In other studies, the number and size of hepatic and splenic MAs have been correlated with levels of environmental contaminants (*37–40*) as well as with aging (*41*). A control population from an area with different contamination levels would be needed to determine whether the increased MA score in older lake whitefish is the result of longer exposure to toxic substances.

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