

## INVESTIGATIONS ON HEALTH OF ANADROMOUS ARCTIC CHAR (*SALVELINUS ALPINUS*) FROM THE EASTERN CANADIAN ARCTIC ECOSYSTEM

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**Keywords:** *Salvelinus alpinus*, histopathology, liver, gills, biomarkers

**Abstract:** The health of the anadromous Arctic char (*Salvelinus alpinus*) in the Eastern Canadian Arctic ecosystem was studied using histopathological features of the liver and gills as biomarkers. Two study sites were sampled in Northern Québec, the George and Sappukait Rivers. Arctic fish livers were characterised by large lipid and low glycogen accumulations. Some hepatic tissue damage was noted in the study fish including the presence of fatty cysts, nuclear inclusions of glycogen, and cellular separation. The gills of the study fish showed no important tissue alterations. The low concentrations of the heavy metals and organochlorines measured in fish muscle samples preclude the establishment of a causal link between these contaminants and the observed histopathological lesions. Based on the histopathological biomarkers in this study, some of the findings suggest that some adverse health effects are occurring.

### ÉTUDE DE L'ÉTAT DE SANTÉ DE L'OMBLE CHEVALIER (*SALVELINUS ALPINUS*) DE L'ÉCOSYSTÈME ARCTIQUE DE L'EST CANADIEN

**Mot clés :** *Salvelinus alpinus*,

**Résumé :** L'état de santé de l'omble chevalier anadrome (*Salvelinus alpinus*) de l'écosystème arctique de l'est du Canada a été estimé grâce à des études histopathologiques du foie et des branchies. Les embouchures des rivières George et Sappukait ont été choisies comme sites d'étude dans le nord du Québec et ce, à cause de leur fréquentation comme sites de pêche par les Inuit pour la rivière George particulièrement, et par les études écologiques à Sappukait, entreprises par le MEF. Les foies des ombles chevaliers des deux sites se caractérisent par des accumulations importantes de lipides et les teneurs faibles en glycogène. Des dommages hépatiques ont été observés, tels des kystes gras, des inclusions nucléaires de glycogène et des séparations cellulaires. Par contre les concentrations faibles de métaux et de contaminants de type organochlorés ne nous permettent pas de conclure à un lien causal entre les anomalies hépatiques et la contamination. Toutefois, en se basant sur les observations histologiques, la santé de l'omble chevalier varie selon l'âge et le lieu d'échantillonnage.

## INTRODUCTION

The presence of contaminants in the Arctic ecosystem was first observed in the early 1970s (Holden, 1972). Contaminants have been found in all the Arctic environmental compartments: air, water, snow and ice (Hargrave *et al.*, 1988), sediments (Muir *et al.*, 1992), suspended particulate matter (Hargrave *et al.*, 1989), biota (fish, marine mammals, polar bears), (Muir *et al.*, 1992), and humans (Charlebois, 1978; Dewailly *et al.*, 1989; Hansen, 1990).

The Arctic environment receives contaminants via the atmosphere and the waterways (Barrie *et al.*, 1992). Some of the contaminants that can be found in this ecosystem are organochlorines (PCB, pesticides, dioxins, and furans), polycyclic aromatic hydrocarbons (PAH), heavy metals (Hg, Pb, As, and Cd), acids (SO<sub>2</sub> and NO<sub>x</sub>) and radionuclides (e.g. <sup>90</sup>Sr and <sup>137</sup>Cs). The physical and biological characteristics of this harsh

environment render it particularly vulnerable to contaminants. Weak sunlight, extensive ice cover, and low temperatures reduce abiotic biodegradative processes (Twitchell, 1991). Furthermore, lipid-rich tissues (> 20% dry weight) typical of arctic fauna contribute to the bioaccumulation and persistence of many lipophilic contaminants (Barrie *et al.*, 1992). These conditions lead to biomagnifications of contaminants up the food chain (Muir *et al.*, 1992). Consequently, Inuit who live in this environment and rely on hunting and fishing practices for subsistence (MacCrimmon & Gots, 1980) can develop health problems following the frequent consumption of contaminated fauna (Bellinger *et al.*, 1987).

This study was part of a large multidisciplinary (social, economical, biological) research project whose mandate

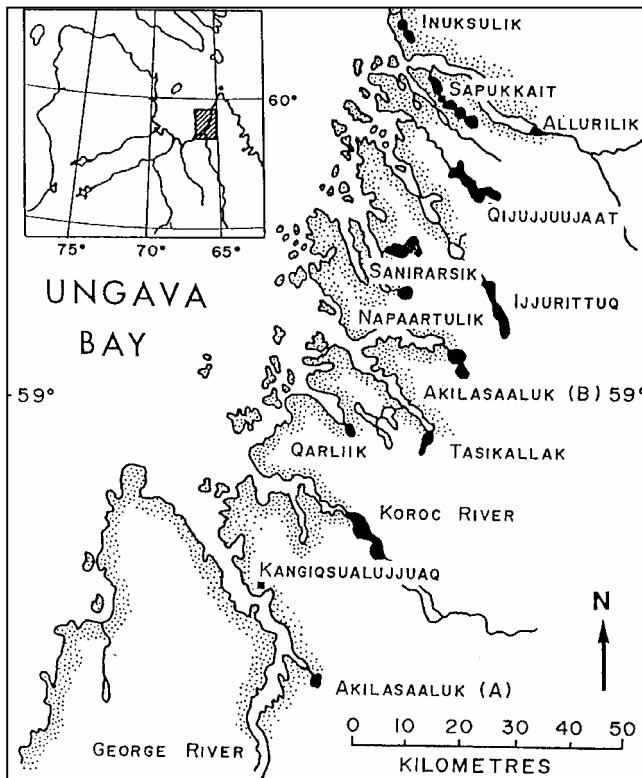


Figure 1. Study sites in the Eastern part of Ungava Bay (Northern Québec, Canada)

was to determine the health status of the Eastern Canadian Arctic ecosystem as a whole. Our specific goal was to determine if there were any effects of contaminants on the health of anadromous Arctic char (*Salvelinus alpinus*), the second most widely consumed species in terms of total weight of traditional food hunted in Northern Québec (Boivin *et al.*, 1989). The nutritional importance of this species for the Northern Québec Inuit renders it an appropriate choice for the examination of possible effects of its consumption on humans. Previous work on the Arctic fauna has focused on determining environmental contaminant levels (Barrie *et al.*, 1992) and body burdens (Muir *et al.*, 1992). However, few studies have attempted to determine the biological effects of the contaminants present (Lockhart & Stewart, 1992).

In this study, the health evaluation of Arctic char was carried out using histopathological biomarkers. These biomarkers respond to a variety of contaminants and are useful in field ecotoxicological studies when multiple contaminants are involved (Hinton *et al.*, 1992). The non-specific response of histopathological biomarkers allows an overall evaluation of an organism's health even though no specific causal contaminants can be identified (Mayer *et al.*, 1992). These results will help determine if contaminants in the Eastern Canadian Arctic ecosystem threaten the health of the aquatic biota and may provide insight to the possible human health risks associated with traditional

diet practices in this ecosystem.

## METHODS

Two different tributaries of Ungava Bay were sampled in the Eastern Canadian Arctic ecosystem, both chosen in collaboration with the Biology Department of the Makivik Corporation and the MEF (Ministère de l'Environnement et de la Faune du Québec). The Sappukait River (site 1) was chosen because a counting dam used by the MEF was in place that facilitated sampling (figure 1). Hydrographical and hydrological information is not available for the Sappukait River, which is actually a series of nine lakes joined by a stream. However, this relatively smaller system is likely to have a smaller drainage basin and flow rate and therefore different hydrodynamic properties. The George River (site 2) was chosen because of its commercial fishery, a factor that was considered important for the Inuit. George River fish were sampled within the estuarine environment (salinity 14). The Sappukait River fish were sampled at its junction with the Alluviaq River (salinity 0). The George River, 475 km in length, is one of the most important rivers reaching Ungava Bay in Northern Québec. Its drainage basin is 41 699 km<sup>2</sup> and the flow rate at the mouth is 871 m<sup>3</sup> s<sup>-1</sup> (Dufour, 1981).

In August 1994, 62 Arctic char were sampled in both study sites, during their upstream migration. At the George River site, three gill-nets were used for sampling. Nets were checked frequently. The 25 captured fish were immediately removed, taken to shore, and placed in transient tidal pools until dissection. Fish remained no more than 10 minutes in either the gill nets or the tidal pools. At the Sappukait River site, 37 fish were trapped in a counting dam, collected, and placed in an enclosure in the river. These fish remained in the enclosure overnight and were sampled the next day. Only live fish were taken for tissue sampling.

All fish were anaesthetised with 3-aminobenzoic acid ethyl ester (1.3 µmol L<sup>-1</sup> of MS-222; Sigma) and sacrificed. Measures of total weight, total length, liver weight, and gonad weight were taken for each fish. The liver and gills of 12 fish of each site were removed and placed immediately into vials containing a solution of 4% formaldehyde-5% glutaraldehyde with 0.1M cacodylate buffer (pH 7.2) for fixation. The otoliths were removed and then the entire fish was frozen. Fixed tissues were kept at 4°C until their return to the laboratory. Note that at the George River site only livers were sampled because of gill damage from the nets. Age determinations using otoliths were performed.

Contaminant measurements were carried out by the

Centre de Toxicologie du Québec. Organochlorines (OC) were measured in muscle samples by high-resolution gas chromatography using an electron capture detector (Dewailly *et al.*, 1991). For these analyses, 9 muscle samples from the Sappukait River fish and 11 muscle samples from the George River fish were dosed. Muscle samples consisted of whole white muscle from the right side of the fish over the lateral line. They were frozen apart in the field and processed as described in Dewailly *et al.*, 1991. The organochlorines measured were  $\beta$ -BHC,  $\alpha$ -chlordane,  $\gamma$ -chlordane, cis-nonachlor, pp'-DDE, pp'-DDT, hexachlorobenzene, mirex, oxychlordane, transnonachlor, and PCBs (aroclor 1260 and congeners 28, 52, 99, 101, 105, 118, 128, 138, 153, 156, 170, 180, 183, and 187). The trace metals measured were cadmium, mercury, lead, and selenium. Mercury was analysed by cold vapour atomic absorption (MEF, 1995a). Cadmium and lead were measured by argon plasma emission spectrometry (MEF, 1995b). Selenium was measured by gas-liquid chromatography analysis as described by Cappon & Smith (1978).

Tissue samples were embedded in two plastic resins, methacrylate and epon, within two weeks of initial fixation. Tissues for methacrylate embedding were dehydrated in methanol (from 50% to 95%) and embedded using a JB-4 embedding kit (Polysciences). Liver sections (1  $\mu$ m) were cut to yield sets of three microscope slides at three different levels. For the gills, sections (1  $\mu$ m) were cut longitudinally to yield three different gill filaments with ten secondary lamellae on either side. Tissues for Epon embedding were post-fixed in 1% osmium, dehydrated in ethanol (from 50% to 100 %) and embedded in Epon 812 resin (MECA Laboratories). Semi-thin sections (0.5  $\mu$ m) were cut for the liver only. For the liver, slides at each tissue level were stained with Lee's methylene blue-basic fuchsine stain and periodic acid Schiff's reagent (PAS) for sections in methacrylate resin and toluidine blue for sections in Epon resin. Staining with Lee's solution allowed an evaluation of general tissue structure and the identification of any lesions present; the PAS stain allowed determination of the relative quantity of hepatic glycogen; and the toluidine blue coloration allowed determination of the relative quantity of hepatic lipid. For the gills, slides with methacrylate sections were stained with Lee's methylene blue-basic fuchsine stain, which allowed an evaluation of the general tissue structure. Neutral lipids were easily recognised in the sections by the shape of the droplet that was different from the droplets of glycogen. Microscopic tissue evaluation was both qualitative (liver and gills) and quantitative (liver). All microscopic analyses were done with a Leitz microscope, coupled with a photographic system

(Nikon camera with WILD MPS 11 adaptor) either for the qualitative analysis or for an image analyser (MOCHA, Jandel) for the quantitative analysis. To ensure objectivity when evaluating each slide, all identifications were hidden and codes assigned. Qualitative analysis began with a survey of the entire slide at low magnification. Individual sections were then examined at higher magnifications. A variety of quality indices were assigned and lesions identified for each tissue. For each study site, 12 fish livers were qualitatively analysed. Each liver sample was assigned qualitative indices at three different tissue levels for three sets of measurements per fish, which were then averaged yielding twelve sets of measurements per site. At the Sappukait River site, ten gill samples were qualitatively analysed, each sample was assigned qualitative indices for ten lamella on three different gill filaments for thirty sets of measurements per fish. Again, values were averaged yielding ten sets of measurements for this site. For both tissue samples, lesions were defined as: 1) alterations of the cell or its compartments; 2) the presence of abnormal structures; 3) cell death; 4) proliferation of cells, connective tissue or bile ducts; and 5) abnormal staining properties. Data were statistically compared between the two study sites using Student's t test.

## RESULTS

Averages of age, weight, length, condition factors (CF), hepato- and gonado-somatic indices (HSI and GSI, respectively), and sex ratios are listed in Table 1. The study fish, aged 6<sup>+</sup> and 7<sup>+</sup>, were in good condition, although most showed no sexual maturation (low GSI). Gonad development was noted in only three females from the George River (GSI= 5.3%, 8.9%, and 2.3%) and condition factors of George River fish were significantly greater than Sappukait River fish. Organochlorine and trace metal concentrations in muscle tissue are listed in Tables 2 and 3, respectively. The organochlorine concentrations were higher in the George River fish. In all cases, the PCBs made up a large proportion of the total organochlorines present in the fish muscle. Cadmium, mercury, and lead concentrations in the fish from the Sappukait and George Rivers were low, with most fish below the detectable levels of the method. Selenium concentrations were the highest but still within the normal range.

The general liver appearance of the Arctic fish was in the mid-range of our tissue quality scale, with the George River fish having the lowest index. The parameters used to assign a quality index to the tissue sections were the general structural organisation and the quantity and intensity of the lesions present. The

Variables measured	Sappukait River n = 37	George River n = 25	Statistical significance
Age yrs	7,1±2,16	7 <sup>+</sup> ± 2,54	p = 0,77
Females:Males	17 : 20	11 : 14	
Weight g	1177 ± 873	1460 ± 654	p = 0,17
Length cm	42,8 ± 12,7	46,5 ± 7,4	p = 0,20
CF <sup>1</sup>	1,16 ± 0,12 <sup>a</sup>	1,26 ± 0,16 <sup>b</sup>	p = 0,005
HSI <sup>2</sup>	2,29 ± 0,64	2,46 ± 0,69	p = 0,32
GSI <sup>3</sup> Females	0,47 ± 0,22 <sup>a</sup>	1,95 ± 2,74 <sup>b</sup>	p = 0,03
GSI <sup>3</sup> Males	0,09 ± 0,04	0,10 ± 0,02	p = 0,16

Table 1. Averages of the morphometric variables, condition factor CF, gonado-somatic index GSI, hepato-somatic index HSI, and sex ratios. Values are arithmetic means and standard errors of the mean. <sup>1</sup>CF: 100 x total weight/ total length<sup>3</sup>. <sup>2</sup>HSI: 100 x weight of liver/ total weight. <sup>3</sup>GSI: 100 x weight of gonads/ total weight. Values identified with <sup>a</sup> and <sup>b</sup> differ each from the other.

Contaminant	Sappukait River		George River	
β-BHC	ND (n = 9)		ND (n = 11)	
α-Chlordane	13.8 ± 1.6 (n = 9)		ND (n = 1)	19.4 ± 11.8 (n = 10)
γ-Chlordane	ND (n = 7)	3.3 ± 0.6 (n = 2)	ND (n = 4)	5.9 ± 3.7 (n = 7)
cis-Chlordane	11.1 ± 1.9 (n = 9)		ND (n = 2)	20.6 ± 18.8 (n = 9)
pp'-DDE	24.1 ± 5.1 (n = 9)		89.9 ± 134.4 (n = 11)	
pp'-DDT	ND (n = 8)	25.0 (n = 1)	ND (n = 7)	94.8 ± 82.7 (n = 4)
Hexachlorobenzene	ND (n = 1)	36.1 ± 5.9 (n = 8)	ND (n = 10)	45.4 ± 15.9 (n = 11)
Mirex	ND (n = 9)		13 (n = 1)	
Oxychlordane	ND (n = 3)	5.1 ± 0.6 (n = 6)	ND (n = 3)	14.3 ± 15.2 (n = 8)
Transnonachlor	19.2 ± 2.2 (n = 9)		ND (n = 1)	44.7 ± 39.3 (n = 10)
PCBs	ND (n = 1)	94 ± 18 (n = 8)	417 ± 637 (n = 11)	
Total Organochlorines	191 ± 30 (n = 9)		677 ± 923 (n = 11)	
% Lipid	8.19		7.87	

ND: not detectable

Table 2: Organochlorine concentrations (ng g<sup>-1</sup> lipid) in Arctic charr muscle samples from the Sappukait and George Rivers.

Metal	Sappukait River (n=9)	George River (n=11)
Cadmium	ND	ND
Mercury	ND	ND (n = 6); 0.15 ± 0.07 (n = 5)
Lead	ND	ND
Selenium	0.83 ± 0.12	0.88 ± 0.15

ND: not detectable.

Table 3: Trace metal concentrations (µg g<sup>-1</sup> dry weight) in Arctic charr muscle samples from the Sappukait and George Rivers.

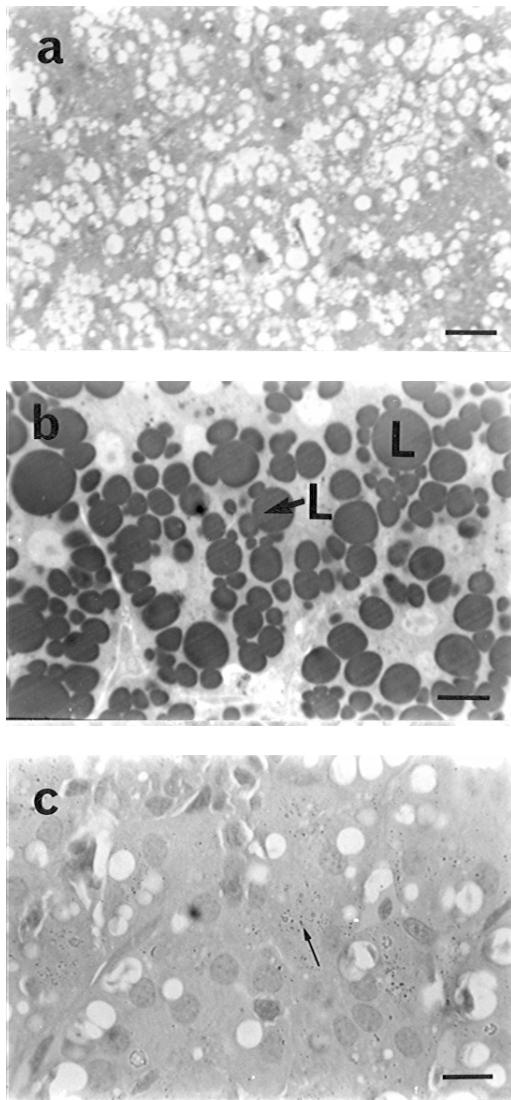


Figure 2. Liver histology.  
**2a** George River fish liver, methacrylate resin, Lee's methylene blue. Note lack of distinct cell cords. Bar = 58  $\mu\text{m}$ . **2b** Fatty liver, Sappukait River fish, epon resin, toluidine blue. L: lipid vacuole. Bar = 12  $\mu\text{m}$ . **2c** Low glycogen content, Sappukait River fish, methacrylate resin, periodic acid Schiff's reagent PAS. Bar = 12  $\mu\text{m}$ .

typical tubular structural arrangement of normal hepatocytes was difficult to observe and the lack of distinct cell cords in some of the fish gave the impression of an unorganized mass of cells (figure 2a). Fatty livers (figure 2b) and low hepatic glycogen content (figure 2c) were frequent observations. Fatty livers were observed in 50% and 45% of the fish from the Sappukait and George River sites, respectively. Although fatty livers were more frequent in the Sappukait River fish, more fish from the George River demonstrated high lipid content at all three of the hepatic tissue levels. Low glycogen content was noted in 75% of the fish from the Sappukait and George Rivers. Sappukait River fish appeared to have the lowest liver glycogen content. Megalocytic hepatitis

was observed in 67% and 83% of the fish from the Sappukait and George Rivers, respectively (figure 3a). George River fish hepatocyte nuclear surface areas ( $38.0 \pm 4.2 \mu\text{m}^2$ ) were significantly larger ( $p < 0.05$ ) than in the Sappukait River fish ( $32.4 \pm 3.8 \mu\text{m}^2$ ). Cell surface areas, however, were not significantly different ( $269 \pm 81 \mu\text{m}^2$  in George River fish compared to  $232.3 \pm 54.3 \mu\text{m}^2$  in Sappukait River fish). Megalocytic hepatitis often co-occurred with focal necrosis. Focal necrosis was noted in 92% and 67% of the fish from the George and Sappukait River sites, respectively (figure 3b). Dilatation of the sinusoids was observed in 67% and 83% of the fish from the Sappukait and George River sites respectively (figure 3c). Other idiopathic hepatic lesions found were nuclear inclusions, fatty cysts, and hepatic cell separation. These alterations were observed in fish from both study sites. Nuclear inclusions were PAS-positive, suggesting the presence of glycogen, and were up to 6  $\mu\text{m}$  in diameter (figure 3d). This anomaly was present in moderate to large quantities in 75% and 50% of the fish from the Sappukait and George Rivers, respectively. Fatty cysts (figure 3e) were observed in Sappukait (17%) and George (42%) River fish, particularly, in livers with extensive lipid accumulation. Cellular separation (figure 3f) was observed in 50% and 33% of the fish from the George and Sappukait Rivers, respectively. This histological alteration was not only more frequent but also more intense in the George River fish.

Gill tissue samples of the Sappukait River fish had intermediate values relative to our tissue quality scale. Some histological anomalies were observed. Although present however, such lesions were rare.

## DISCUSSION

The assessment of ecosystem health has become an important issue in recent years (Holdway *et al.*, 1995). More methods are being developed and improved to properly evaluate the effects of environmental contaminants (Adams & Ryon, 1994). One method that has received considerable attention is the biomarker approach (Stein *et al.*, 1992; Brumley *et al.*, 1995; Soimasuo *et al.*, 1995; Lagadic *et al.*, 1997). Biomarkers are biological parameters used to evaluate the effects of environmental contaminants on organisms (Adams, 1990). The use of biomarkers has been suggested as a promising way to determine whether contaminants transported to the Arctic ecosystem induce adverse health effects in the Arctic biota (Lockhart, 1995). In this study, the health of anadromous Arctic char from the Eastern Canadian Arctic was assessed using biomarkers at the organism and tissue levels.

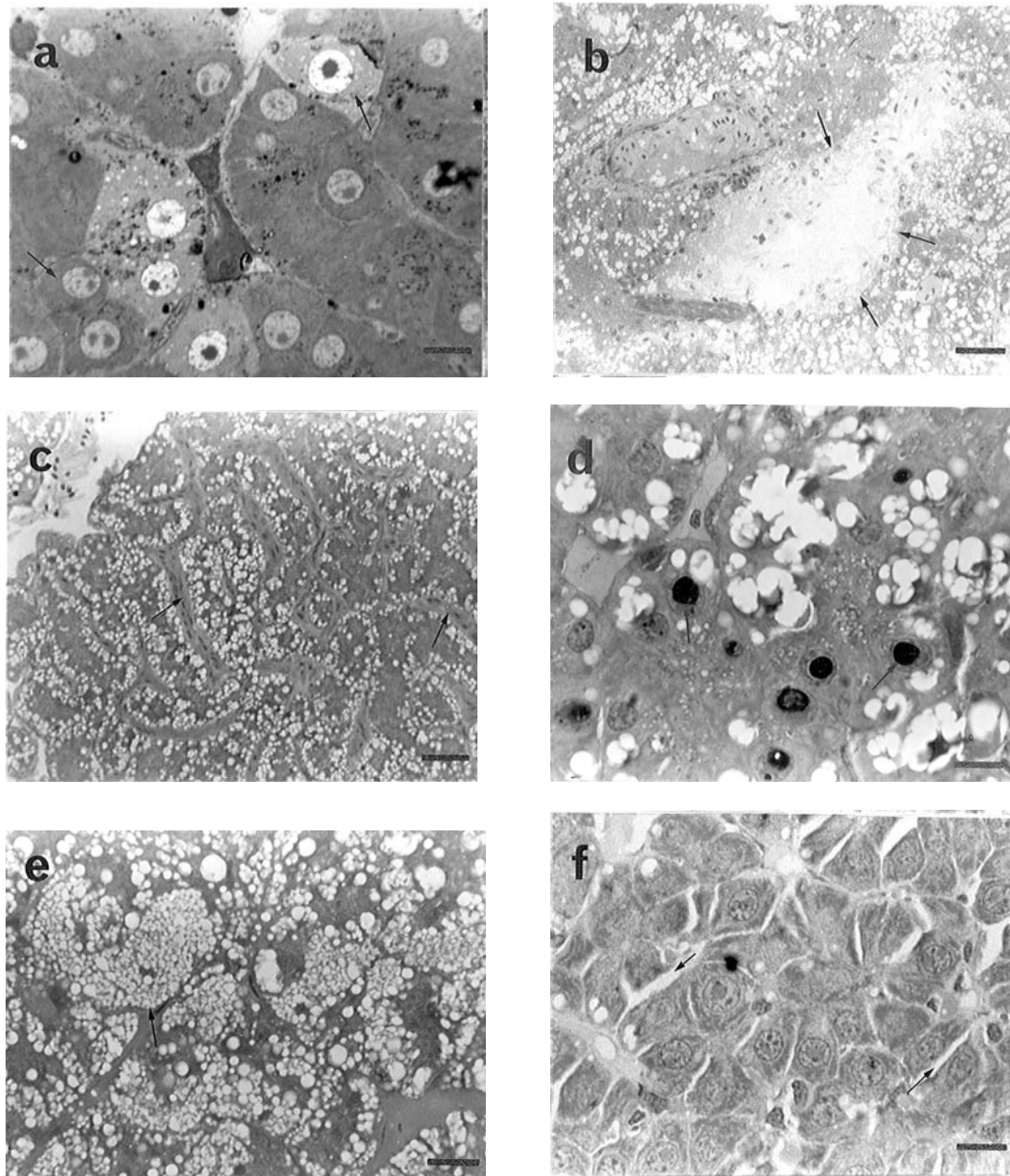


Figure 3. Liver histological features observed.

**3a** Megalocytic hepatosis, George River fish, epon resin, toluidine blue. Note enlarged pale hepatocyte with enlarged pale nucleus compared to normal hepatocyte with normal nucleus arrows. Bar = 10  $\mu$ m. **3b** Focal necrosis, George River fish, methacrylate resin, Lee's methylene blue. Note large area of cell death arrows. Bar = 50  $\mu$ m. **3c** Sinusoidal dilation arrows, Sappukait River fish, methacrylate resin, Lee's methylene blue. Bar = 50  $\mu$ m. **3d** Nuclear inclusions of glycogen arrows, Sappukait River fish, methacrylate resin, PAS. Bar = 10  $\mu$ m. **3e** Fatty cysts arrows, George River fish, methacrylate resin, Lee's methylene blue. Bar = 50  $\mu$ m. **3f** Hepatic cell separation, George River fish, methacrylate resin, Lee's methylene blue. Note enlargement of the intercellular space arrows. Bar = 10  $\mu$ m.

Organismal indices (CF, HSI, and GSI) are relatively simple and provide rapid indications of an individual's health status (Goede & Barton, 1990). The CFs of the fish from both study sites were always above unity, indicative of good health and comparable to other Arctic char condition factors reported in the literature (Boivin & Power, 1990). The higher CFs of the George River fish can be explained by the stomach contents of the fish. The stomachs were full in the George River

fish (average stomach content wt. 59.4 g) while relatively empty in the Sappukait River fish (average stomach content wt. 1.6 g). During upstream or spawning migrations, salmonids generally cease feeding (Stolz & Schnell, 1991). The fish at the Sappukait River site had already reached freshwater and may therefore have ceased feeding before the George River fish, which were sampled in the estuary. No differences were observed between the hepato- and

gonado-somatic indices of the study site fish. Little or no sexual maturation had occurred at either site, although three of the female George River fish demonstrated gonad development (GSI 5.3%, 8.9%, and 2.3%). Arctic char, which generally spawn every 2 or 3 years due to energetic constraints (Dutil, 1986), may not migrate during a reproductive year, or reproducing individuals may migrate one month prior to non-reproducing individuals (Johnson, 1980). This may explain the lack of sexually mature fish in our samples.

In field investigations, histopathological biomarkers are useful in evaluating contaminant exposure due to their non-specific response to a variety of contaminants present in low concentrations (Hinton & Laurén, 1990; Niimi, 1990). They reflect biochemical and physiological alterations and can, to a certain degree, be related to higher levels of biological organisation (Adams, 1990; Meyers & Hendricks, 1985) allowing some long-term predictions. The use of histological indices, however, requires a good knowledge of that species' tissue histological appearance since a variety of abiotic (salinity, temperature) and biotic (age, sex, reproduction) parameters can cause alterations in appearance. In the Arctic, contamination is diffuse and widespread, so no specific sites can be used as controls. In the livers of Arctic char from the Sappukait and George Rivers, a variety of histological alterations were observed. Three were of special interest and present at both study sites. These anomalies were fatty cysts, cellular separation, and nuclear inclusions of glycogen.

Fatty cysts are aggregations of lipid vacuoles from two or more different hepatocytes following their rupture (Rouiller, 1964). The lipid vacuoles from the burst cells flow into the extra-cellular space and generally induce connective tissue proliferation. In our fish, the observed lipid vacuole aggregations did not induce this proliferation. Fatty cysts, present in the study fish, were observed mainly in livers with elevated lipid content. This lesion could be caused by an increase in the cell's internal pressure, due to extensive lipid accumulation, resulting in the rupture of cells. In the literature, fatty cysts have not been reported following contaminant exposure. However, choline deficiency is known to induce this histological liver alteration in sturgeon (Hung, 1989). Choline molecules are a part of the phospholipids that make up biological membranes. This supports the hypothesis that some factor is interacting with the biological membranes, creating a weakness and facilitating cell rupture.

Cellular separation is an enlargement of the intercellular space and occurs when cell-cell adhesion is lost. Cell-cell contact, which is fundamental for tissue structure maintenance and the physiological

functioning of the cells, is achieved by adhesion molecules or molecular complexes (proteins with -SH groups) and depends upon calcium content and temperature (Öbrink *et al.*, 1980). An interference with the calcium flux to the cells or an alteration in the adhesion molecules or molecular complexes may induce this lesion. Mercury exposure has been shown to decrease the liver calcium content in the teleost *Notopterus notopterus* (Verma & Tonk, 1983) and induce an enlargement of the intercellular space in *Channa punctatus* (Sastry & Gupta, 1978). However, cell separation in the latter study was thought to be a consequence of the breakdown of connective tissue.

Nuclear inclusions of glycogen, which may be observed at low frequencies (< 0.1% of nuclei) in normal mammalian livers (Reid, 1973), are considered pathological when present in large quantities (Sparrow & Ashworth, 1965). In the Arctic fish, this lesion was observed in 75% and 50% of the Sappukait and George River fish, respectively, in moderate to high frequency. This particular anomaly can be observed at high frequencies in mammals with glycogen storage diseases such as diabetes (Carami *et al.*, 1968) or Von Gierke's disease (Sheldon *et al.*, 1962). In fish, no pathological investigations have reported nuclear inclusions of glycogen to be a consequence of similar diseases. One contaminant known to induce this lesion in fish is diethylnitrosamine; however, the synthetic pathways of nuclear glycogen as well as the significance of these inclusions are unknown (Thiyagarajah & Grizzle, 1985).

To our knowledge, in the marine environment, fatty cysts, cellular separation, and nuclear glycogen inclusions observed in the George and Sappukait River fish have not been reported to occur under normal conditions. However, in acidic lakes, Arctic char, has been shown to store glycogen in hepatocyte nuclei and to develop nuclear pathology (Hofer *et al.*, 1997).

Other hepatic abnormalities were also found in the fish from both the Sappukait and George Rivers: liver cord disarray, fatty livers, low glycogen content, megalocytic hepatitis, necrosis, and sinusoidal dilation. Liver cord disarray is the consequence of a rupture and/or a degeneration of the connective tissue (Sastry & Gupta, 1978). Some contaminants are known to induce this histological change in fish, for example, Hg in *Channa punctatus* (Sastry & Gupta, 1978) and Pb in *Puntius arulius* (Bengeri & Patil, 1986). In mammals, however, genetic disorders and malnutrition (lack of vitamin C) can induce connective tissue alterations (Levene, 1980). Also, increasing age has been associated with a degeneration of the liver structure in flounder (*Platichthys flesus*) (Peters *et al.*, 1987). At the George River site, the oldest fish, aged 14<sup>+</sup> and 11<sup>+</sup>, had the lowest tissue quality index, which

reflects the overall tissue appearance and the extent of hepatic histological alterations. In these older fish, age may have been responsible for the liver cord disarray observed.

Abnormal accumulations of hepatic lipids can be caused by a variety of contaminants such as Hg in *Fundulus heteroclitus* (Weis *et al.*, 1986) and PCB in *Ictalurus punctatus* (Lipsky *et al.*, 1978). This pathological condition results from impaired utilisation and/or synthesis of hepatic lipids (Lombardi, 1966). Contaminants likely interfere with the production of lipoproteins (*i.e.* the exportable form of hepatic lipids (Sheridan, 1988)) by altering ribosomes and affecting protein synthesis (Cheville, 1988). However, normal seasonal increases of hepatic lipid content have been shown to occur in the natural environment in *Oncorhynchus keta* (Hatano *et al.*, 1989) and when the liver is the main site of lipid storage, as in cod (Morrison, 1987). Arctic char, however, tend to store their lipids in the abdominal cavity (Jensen, 1980).

The relative absence of glycogen in the study fish may be a direct consequence of the high energy requirement imposed on the fish during the migration period as these fish were sampled during their upstream migration. Carbohydrates are known to be the primary source of energy for teleosts under stressful conditions (Dangé, 1986) and for activities such as migration (McKeown, 1984).

Megalocytic hepatitis and hepatic focal necrosis can be indicative of contaminant exposure. Megalocytic hepatitis has been considered an indicator of exposure to contaminants in flat fish (Myers *et al.*, 1991) and Atlantic salmon (Kent *et al.*, 1988). However, this lesion can also be induced by natural toxins (Wales, 1967) and has been reported in fish from uncontaminated waters, although in low frequencies (Malins *et al.*, 1984). Necrosis is often encountered in fish exposed to contaminants (e.g. DDT and malathion: *Sarotherodon mossambicus* (Ramalingam, 1988)). Other factors such as age (Peters *et al.*, 1987) and parasite load (Grizzle & Kiryu, 1993) can also induce necrosis in fish livers. In Northern Québec and Labrador, Arctic char average life expectancy is 15-16 years (Boivin *et al.*, 1989; Scott and Scott, 1988), although individuals of this species can live up to 40 years (Hunter, 1976). In this study, a 14 year-old individual from George River had a high necrosis index. Old age in this individual may have caused the extensive tissue necrosis observed. However, at the Sappukait River site, a 4<sup>+</sup> fish also had a similar necrosis index. A parasitological study conducted on the same study fish revealed few hepatic parasites (Desdèves *et al.*, 1998), and their presence was not correlated with the extent of necrosis encountered.

Gills were the second organ examined for histological

alterations. The gills of the Sappukait River fish are in relatively good condition and no extensive histological alterations were observed. Although some histological anomalies were detected, they were only present in a few of the secondary lamellae. We conclude that no adverse health effects are reflected in the gills of the Arctic fish.

Concentrations of the trace metals Pb, Zn, and Hg measured in the study fish were low. Although Hg can induce some of the hepatic and gill histological lesions observed in the study fish, the frequency and intensity of these lesions was not correlated with the muscle burden of this contaminant. In addition, these values are comparable to Hg tissue concentrations observed in fish from natural unperturbed systems in the James Bay area, which are generally less than 0.2 µg g<sup>-1</sup> wet weight (Messier *et al.*, 1985). Lead concentrations (0.03 µg g<sup>-1</sup> dry weight) were also in the range reported in uncontaminated fish muscles (< 0.1 µg g<sup>-1</sup> dry weight) (Cossa *et al.*, 1993). Higher concentrations of muscular selenium than those of Hg and Pb. This is not unexpected since Se is an essential element with an antioxidant role (Ashley, 1972). However, the observed concentrations were lower than those that have been reported in fish from uncontaminated waters (Ohlendorf & Hothem, 1995). The low trace metal muscle burdens and the absence of a relationship between the metal concentrations and the frequency and intensity of the different histopathological alterations observed preclude the suggestion of a causal relationship between the lesions and contamination by heavy metals. However, it has been recently observed that tissue alterations could be observed even with low concentrations of trace metals (Jeantet *et al.*, 1997)

The different organochlorine concentrations measured in the muscle samples of the study fish were low. Concentrations of PCBs and HCBs in English sole (*Parophrys vetulus*) muscle from Puget Sound have been reported to be 3.4 µg g<sup>-1</sup> dry wt and 0.17 µg g<sup>-1</sup> dry wt, respectively (Malins *et al.*, 1984). In comparison, the highest measured concentration of PCBs and HCBs in the Sappukait fish were ca. 0.07 and 0.01 µg g<sup>-1</sup> dry weight (lipid muscle content in Sappukait fish = 8% of wet weight), respectively. Fish near a sewage outlet in Sidney (Australia) had average pp'-DDE and pp'-DDT muscle concentrations of 0.95 and 0.27 µg g<sup>-1</sup> wet wt, respectively (Miskiewicz & Gibbs, 1994). These concentrations of PCB, HCB, pp'-DDE and pp'-DDT are more than tenfold higher than in fish from the Eastern Canadian Arctic ecosystem. Although levels were low in all fish, the George River fish always had the highest organochlorine concentration in the muscle, perhaps because of their higher mean age.

Many field investigations have used histopathological



biomarkers as health indicators in fish from polluted waterways (Bucke & Feist, 1993; Khan *et al.*, 1994; Malins *et al.*, 1985; Pierce *et al.*, 1978). Generally, these studies indicate that fish from contaminated sites demonstrate more histological anomalies and at higher frequencies than fish from the reference sites. These results suggest that exposure to contaminants in the environment increases the frequency of histological lesions inducing adverse health effects in these fish. However, no clear cause and effect relationship between contaminant presence and histopathological effects can be established since the specific causes of histological lesions are not always identified (Bowser *et al.*, 1990).

In our study, contaminant aetiology for the observed effects is also difficult to establish. Although histopathological studies often have small sample sizes (Dutil *et al.*, 1992), this investigation of the health of Arctic char in the Eastern Canadian Arctic ecosystem has allowed us to draw some conclusions. The difficulty related to field studies in the Arctic must be taken into consideration in regard to the number of fish that can be sampled due to quotas allowed by Inuit. However, our data set shows clearly that some adverse health effects are present in Arctic char of this ecosystem. These were reflected mainly in the liver. Three lesions were specifically considered indicators of health effects in the Arctic fish: fatty cysts, nuclear inclusions of glycogen, and cellular separation as these lesions are not known to occur under normal conditions. The presence of more important histological anomalies in the liver suggests that if a causal connection exists between contaminants and anomalies, the food chain would appear to be a more important contamination route than direct water contact. Further research using biochemical and reproductive biomarkers is suggested. These health indicators should help understand if and how contaminants are interacting with Arctic char in this ecosystem and determine what the possible long-term ramifications could be at the population and community levels.

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