RECENT CANADIAN STUDIES ON THE PHYSIOLOGICAL EFFECTS OF PULP MILL EFFLUENT ON FISH

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CANADA'S GREEN PLAN
LE PLAN VERT DU CANADA

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INTRODUCTION

In December 1991, the federal government announced a Pulp and Paper Regulatory Package that included regulations on the discharge of BOD, suspended solids and acute toxicity for all pulp and paper mills, as well as placing strict limits on discharges of chlorinated dioxins from pulp mills employing bleaching. These new regulations address concerns regarding the potential discharge of persistent, bioaccumulating substances, and they reduce the impact of aquatic discharges on the receiving environment.

The federal package did not include a regulatory limit on chlorinated organic compounds (other than dioxins). Several countries, including Denmark, Sweden and Finland currently regulate these compounds in pulp mill effluents using the sum parameter Adsorbable Organic Halogens (AOX). AOX is used as a sum parameter because it measures the total amount of chlorine associated with organic compounds in effluent. The objective of these AOX regulations was to control the sublethal effects in fish observed around pulp mills in Sweden that had been associated by the Swedish scientists with AOX. However, in 1989-1990, an assessment of effluent from pulp mills using bleaching was undertaken under the Canadian Environmental Protection Act. The assessment report could not establish a scientific basis for setting a federal AOX level (Environment Canada, 1991). The assessment panel concluded that AOX as a measurement did not provide an estimate of environmental toxicity, persistence or bioaccumulation. For example, Martinsen *et al.* (1988) estimated that only 0.1% of AOX in pulp mill bleach liquor could be expected to bioaccumulate.

During the development of the regulatory package, recent Canadian research confirmed that impacts on wild fish in the receiving environment were associated with the discharge of effluent from Canadian pulp mills. These effects relate to physiological responses of fish to effluent exposure and include changes in the ability of the fish to control their production of reproductive steroid hormones. These changes have been correlated with decreased reproductive performance. However, the absence of a correlation between AOX production by pulp mills and these physiological disruptions resulted in a decision of the federal government not to include an AOX regulation in the December 1991 regulatory package. The federal government has set a high priority on identifying the compounds that are responsible for the damage detected in wild fish and on identifying pulping or waste treatment processes that eliminate these effects.

The purpose of this document is to review the data that prompted the federal government studies, and to outline the recent, significant findings on effects of pulp mill discharges in Canadian environments.

OVERVIEW OF PREVIOUS WORK

Most field studies of chronic effects of pulp mill effluent conducted prior to 1985 focused on benthic communities through the use of community diversity indices and abundance of indicator species. The results of these studies were reviewed by McLeay (1987) and Owens (1991). Although effluent impacts have been observed, they appear to be due to fibre mats, colour, turbidity, and low dissolved oxygen conditions caused by decomposition of organic substances. In cases where the data set included chemical and toxicological characterization of the effluents, the observed effects were consistent with organic and nutrient enrichment impacts (Owens, 1991). One field study that compared concentrations of chlorinated organic compounds with impacts on benthic species concluded that there was no correlation between AOX exposure and effects (Craig *et al.*, 1990).

In the past, impacts on fish and fish populations of effluents were assessed by measurement of their acute lethality. The major contributors to toxicity of whole mill effluents are resin acids and other extractives present in cooking liquors (Rogers & Mahood, 1974; McLeay *et al.*, 1979a & 1979b: Kovacs, 1986). These substances are generally amenable to detoxification by secondary treatment (Servizi *et al.*, 1986). Detectable levels of individual chlorinated compounds are present in specific waste streams such as spent bleachery effluents from the C- and E- stages of bleaching. However, the contribution of chlorinated compounds to toxicity of whole mill effluents is debatable, since there appeared to be no marked difference in toxicity of bleached versus unbleached pulp mill effluent (Mehrle *et al.*, 1989) or correlation between AOX and final effluent toxicity (Craig *et al.*, 1990; O'Connor *et al.*, 1993). According to Folke (1991), spent bleach liquors are seldom acutely toxic to organisms. The recent amendments to the Fisheries Act (Pulp & Paper Regulatory Package) require that all discharged effluents be non-toxic.

Until recently, there has been little published information on the sublethal effects of pulp mill effluent on wild fish populations. The Swedish "Environment/Cellulose I Project" was an innovative study of the physiological, pathological and ecological impacts of primary treated effluent from a bleached kraft mill on the fish communities of the Gulf of Bothnia (Södergren *et al.*, 1988; Södergren, 1989). The fish population was sampled on four occasions from May 1984 to September 1985, at sites 2, 4.5, 8, and 10 km from the discharge of the mill at Norrsundet. An unexposed reference site was sampled about 120 km north of the discharge point. Fish density and diversity were low in areas less than 2 km from the discharge (Neuman and Karås, 1988). At sites 2 km to 4.5 km from the discharge, densities of both adults and fry of roach (*Rutilus rutilus*) and ruffe (*Gymnocephalus cernua*) increased dramatically, an effect that the authors attributed to effects of nutrient enrichment by the effluent. In contrast, the abundance of adult and pelagic fry of perch (*Perca fluviatilis* L.), sand-goby (*Pomatoschistus minutus* (Pallas)), and herring (*Clupea harengus*) were low at the 2 km and 4.5 km sites, increasing in abundance at sites further from the discharge. A number of skin diseases and skeletal deformities were also noted, including deformed gills, skulls, fins, and spinal columns, and skin sores, fungal infections, and fin erosions (Bengtsson, 1988). The prevalence of these diseases was particularly high in 1984, and seemed to be exposure-dependent, i.e., more frequent at sites nearest the discharge where effluent concentrations were highest. Relative to the reference area, perch near the Norrsundet mill had smaller gonads, larger livers, higher activities of liver enzymes, increased liver ascorbic acid content, marked changes in energy storage and use, impaired osmotic and ion regulation, increased red blood cell counts and haematocrits, and reduced lymphocyte counts (Andersson *et al.*, 1988; Larsson *et al.*, 1988; Sandström *et al.*, 1988). The increased activity (induction) of liver enzymes (7-ethoxyresorufin-O-deethylase (EROD), one of the mixed function oxygenases (MFOs)) was interpreted as a specific response to chemical exposure.

The MFO response is the measurable increase in activity of liver enzymes, usually EROD or aryl hydrocarbon hydroxylase (AHH) (Hodson *et al.*, 1991a). These enzymes increase in activity because more enzyme is produced by cells in response to exposure to families of compounds such as non-chlorinated polynuclear aromatic hydrocarbons, plant flavones, chlorinated dioxins and furans, polychlorinated biphenyls and chlorodiphenylethers. Only some members of these families of compounds can cause an increase in activity (enzyme induction), since they must have a very specific molecular size, shape and conformation to interact with the cell receptor that turns on enzyme production. Since these compounds are also highly toxic, an increase in MFO activity is interpreted as both chemical exposure of the organism and as possible toxicity. The consistent occurrence of MFO activity in fish populations exposed to pulp mill effluent, usually in concert with other physiological, pathological and population changes, has stimulated its use as an indicator of the presence and possible effects of pulp mill effluents. Since MFO activity increases within 24-48 hours of exposure to pulp mill effluent, it provides a rapid indicator of possible effects long before other symptoms of toxicity appear. Consequently, it is a useful tool in laboratory and field experiments for evaluating the relative potency of pulp mill effluents and their components.

In the Swedish studies, many of the physiological changes also followed an exposure-response relationship, with responses most pronounced at the two sites nearest the discharge (2 and 4.5 km, representing effluent dilution factors of 160 and 330-fold, respectively). However, some response (reduced gonad size, EROD induction, increased red blood cell count, decreased lymphocyte count, and poor chloride regulation) still occurred 10 km from the mill, at a dilution factor of more than 1100-fold.

To compare the effects of unbleached vs. bleached effluent, an additional site was selected in the receiving waters of an unbleached kraft mill at Sandarne. In contrast to the perch sampled near Norrsundet, Sandarne perch showed no effects or only small effects for most parameters measured. Södergren (1989) regarded this as evidence of a relationship between the bleaching process (i.e., levels of chlorinated organic compounds) and subsequent effects in the receiving water. These conclusions were cited as the basis for using AOX to regulate pulp mills in Denmark, Sweden and Finland (Folke, 1991).

When these results were originally released, their applicability to the North American situation was questioned by some scientists, particularly since North American studies had not detected the responses seen in the Baltic. A "Scientific Panel on Pulping Effluents in the Aquatic Environment" was convened to address the discrepancies between the Swedish and North American studies (Eysenbach *et al.*, 1990). Among its conclusions (Merhle *et al.*, 1989) was the assertion that Swedish studies should not be extrapolated directly to North American mills, for several reasons:

- many physical, chemical and biological differences exist between Swedish and North American situations;
- the Gulf of Bothnia is a unique hydrological system, unrelated to the receiving waters of North American mills;
- secondary biological treatment of effluents at many North American mills effectively reduces or eliminates sub-lethal effects in the receiving environment.

In addition, the comparison between Swedish mills with and without chlorine bleaching was considered inappropriate. Compared to the bleached mill, the unbleached mill had a lower pulp production and a lower BOD loading, combined with a greater flushing and effluent dilution in the receiving water. Hence, fish near the unbleached mill were exposed to lower concentrations of effluent than those at the bleached mill. The panel concluded that it was not possible to draw cause-effect inferences about chlorinated organic compounds from comparisons between the two cases, and that the toxicological database then available on the fate and effects of compounds in pulp mill effluents needed to be expanded. In particular, assessments of impacts of pulp mill effluents required the integrated use of chemical analyses, ecological field assessments, and toxicity testing to document the source of the adverse effects.

Studies in the United States by the National Council of the Paper Industry for Air and Stream Improvement (NCASI) supported the contention that secondary-treated bleached kraft mill effluent had little or no detrimental effect on receiving water ecosystems. The first of these studies was centred in New Bern, North Carolina, and represented a southern U.S. "warm-water" stream ecosystem (NCASI, 1984). Concentrations of up to 18% v/v of secondary-treated effluent entered two artificial streams, while two control streams received only river water. Production, growth, survival, and reproduction of largemouth bass (*Micropterous salmoides*), bluegill sunfish (*Lepomis macrochirus*), and golden shiner (*Notemigonus crysoleucus*) were monitored, along with a series of other ecological and physical/chemical parameters. Observed effects were less pronounced and occurred at higher effluent concentrations than effects noted in the Swedish studies. However, the most sensitive response in the Swedish studies, increased activity of the MFO system, was not measured. At an effluent concentration of 18% v/v, largemouth bass production (total weight gain of all fish) was decreased relative to the control stream, but there were no other significant effects in bass, nor were there effects noted on the other two fish species studied. At an effluent concentration of 10% v/v, there were no effects reported for all three fish species. Although, the early studies in artificial channels did not show any significant responses in fish, they did not examine many of the sublethal effects reported by the Swedish studies. Recent studies using these artificial streams have incorporated the determination of MFO activity in fish. EROD activity and P4501A1 concentration were recently reported to be elevated in both bass and catfish exposed to 8 % effluent in artificial streams (Borton, 1992)

A parallel "cold-water" study, using rainbow trout (*Oncorhynchus mykiss*) was conducted at Lewiston, Idaho from 1980 to 1988 (NCASI, 1989; Hall *et al.*, 1991). The first part of the study increased the effluent concentration in the treated streams at about 10-month intervals from 1.3% v/v to 5.1% v/v. In the second part, effluent concentration was held at about 1.5% v/v for 3.5 years, and rainbow trout were monitored throughout their entire life cycle. At all effluent concentrations, trout from the two treated streams generally showed higher growth rates, total production, and average weight than fish in control streams, while survival seemed somewhat reduced by effluent exposure. There was no significant relationship between effluent exposure and liver size, blood haematocrit or leucocrit, or prevalence of histopathological lesions. Limited spawning occurred in both control and treated streams, but it was impossible to determine whether treatment differences in hatchability were significant due to small sample sizes. Total weight, final mean weight, mean weight gain, and total weight gain were significantly greater for fry from the treated streams than for fry from the control streams, as observed in ruffe in Sweden.

A comprehensive review of the effects of pulp mill effluents in the aquatic environment has recently been published. Owens (1991) concluded that no complete mechanistic link had been demonstrated that relates exposure to contaminants in pulp mill effluents to "within-organism" responses, "whole-organism" effects and effects at the population and community level. He called for a multidisciplinary effort, to combine chemical, toxicological and biological studies of adverse effects and to identify the responsible chemicals.

RECENT CANADIAN STUDIES

Recent Canadian studies have tested whether effects, particularly those seen in Sweden, can be found in wild fish near Canadian mills producing bleached kraft pulp. When effects were found, further studies looked for effects at mills with different production or waste treatment technologies.

Are sublethal effects present in fish from Canadian waters receiving bleached kraft pulp mill effluent?

Two Canadian field studies have examined fish in waters receiving effluents from pulp mills employing technologies similar to that of the Norrsundet mill that was the major focus of the Swedish "Environment/Cellulose I Project". These Canadian studies were located in Jackfish Bay, Ontario, on the north shore of Lake Superior, and in the St. Maurice River, Quebec. Both of these mills utilized only primary treatment of effluent at the time of these studies (1988-1989).

The Lake Superior project was prompted by a 1987/88 study by the Ontario Ministry of the Environment in which liver MFO enzymes increased in fish near a 1200 tonne/day bleached kraft pulp mill discharging primary-treated effluent to Jackfish Bay (Smith *et al.*, 1991). Beginning in 1988, a variety of responses were investigated in three species of fish. Compared to the reference site, white sucker (*Catostomus commersoni*) from Jackfish Bay exhibited higher condition factors, slower growth rates, increased age to maturity, smaller gonads, smaller egg size (females) and fewer secondary sexual characteristics (males), increased liver MFO activity, and lower levels of serum sex hormones (Munkittrick *et al.*, 1991; McMaster *et al.*, 1991, 1992). Increased liver MFO and decreased serum sex hormones were also observed in longnose sucker (*Catostomus catostomus*) and lake whitefish (*Coregonus clupeaformis*) (Munkittrick *et al.*, 1992a, 1992b).

Increased levels of MFO activity have also been found at other kraft mills in Ontario, Quebec, B.C. and Alberta. In concert with increased MFO activity, several species of fish exposed to pulp mill effluent experienced severe impacts on steroid hormone levels. At Jackfish Bay, decreases in circulating levels of gonadal steroid hormones were associated with reductions in secondary sexual characteristics and gonadal size, and increases in age to maturity (Munkittrick *et al.*, 1991). Similar changes in sex steroid metabolism are known to occur in mammals exposed to compounds that also induce the MFO system.

The St. Maurice River study focused on white sucker and to a lesser extent northern pike (*Esox lucius*). Fish were collected at sites upstream and downstream from a hydroelectric dam and a 1400 tonne/day bleached softwood kraft mill located at La Tuque, Quebec, that discharged primary-treated effluent into the river. Downstream, white sucker showed increased liver MFO activity (Fig. 1a), larger livers (Fig. 1b), signs of non-specific stress, changes in serum concentrations of reproductive steroids, and subtle population changes. These responses were greatest immediately downstream from the mill and recovered progressively towards control values at sites further downstream (Hodson *et al.*, 1991b; 1992). The effects paralleled exposure to pulp mill effluents contaminants as indicated by concentrations of chlorinated dioxins in fish tissue and chlorinated phenolics in the water; both chemical contamination and MFO activity were still significantly elevated at a site 95 km downstream from the mill. However, there was no correlation between the gradient of AOX levels in river water and the gradient of responses in fish.

These studies indicate that effects similar to those observed in Sweden are found at Canadian sites.

Do bleached kraft mills with modernized processes show the same effects?

'Modern' mills are those that substitute chlorine dioxide (ClO_2) for molecular chlorine (Cl_2) as a bleaching agent, and which may use oxygen or peroxide as additional bleaching agents. Modern mills usually have better in-plant waste recovery, more efficient pulping systems, as well as full secondary treatment of effluent (Berry *et al.*, 1991).

Recent Canadian studies indicate that some effects on fish can also be found in wild fish exposed to treated effluent from modern or modernized bleached kraft mills. The sites of these studies include: Jackfish Bay after the initiation of secondary treatment (Munkittrick, 1992; Munkittrick *et al.*, 1992a, 1992b; Van Der Kraak *et al.*, 1992); the Spanish River, Ontario (Servos *et al.*, in press); the Wapiti River, Alberta (Kloepper-Sams *et al.*, 1992a,b; Swanson and Kloepper-Sams, 1992); and the Fraser River, British Columbia (Rogers *et al.*, 1989). The most consistently observed response in all studies was increased liver MFO activities, associated with exposure of white sucker, longnose sucker, lake whitefish, chinook salmon (*Oncorhynchus tshawytscha*) and mountain whitefish (*Prosopium williamsoni*) to secondary-treated bleached kraft mill effluents.

Munkittrick *et al.* (1992a, 1992b) monitored three fish populations in Jackfish Bay and failed to detect improvements in MFO induction and plasma sex steroid effects for three years following the installation of secondary treatment at the Terrace Bay mill in 1989. However, physiological effects are alleviated during mill operational shutdowns (Munkittrick *et al.*, 1992a) and are evident in fish exposed to effluent in the laboratory (Williams *et al.*, 1992). The continued presence of effects suggests that the causative agents are not removed during secondary treatment.

Kloepper-Sams *et al.* (1992) did not find dramatic effects on fish downstream from a modernized bleached kraft mill in Alberta, other than increased levels of tissue dioxins and increased MFO activity. The mill was located on the Wapiti River which has no dams to impede fish movement. Extensive fish migrations observed during the study may have confounded comparisons among exposed and unexposed sites, since the exposure of fish to effluent would be highly variable over time. Servos *et al.* (in press) studied the impact of the discharge of a 1200 tonne/day bleached kraft mill employing oxygen delignification, partial ClO_2 substitution and secondary treatment on white sucker populations in the Spanish River, Ontario, downstream from the mill and an associated dam. Liver MFO activities were elevated up to 51 km downstream from the mill and liver sizes were increased immediately below the outfall (Fig. 2). Gonad sizes and plasma sex hormones were low at all sites including controls because the fish were still recovering from spawning at the time of sampling. White sucker sampled later in the season during the following year showed steroid hormone changes immediately downstream from the mill, consistent with those observed in Jackfish Bay and the St. Maurice River (Munkittrick *et al.*, MS).

The mills at Jackfish Bay and on the Spanish River discharged less than 1.6 kg AOX /ADMT of pulp. Recent studies on the Spanish River shows that induction is still present at AOX discharges below 0.7 kg/ ADMT (Servos and Munkittrick, unpubl. data). These studies confirm that modernized mills with secondary treatment, which are discharging low levels of AOX, are capable of producing the same physiological disruptions evident at sites where the mills employed older technology.

Are sublethal effects present at non-kraft mills or mills with no bleaching?

In the summer of 1991, samples were collected at 12 sites in Ontario for laboratory toxicological testing, assessment of receiving water quality, and responses of fish to pulp mill effluent. Sampling was generally restricted to one species (the white sucker) and small sample sizes (n=12 for each sex) to provide preliminary indication of effects and to focus subsequent follow-up studies. These data have been presented at a number of international scientific conferences, including the international Association for Great Lakes Research (Waterloo, June 1992), a joint meeting of SETAC Europe and the Aquatic Ecosystem Health and Management Society (Potsdam, Germany, June 1992), the Aquatic Toxicity Workshop (Edmonton, Alberta October 1992), SETAC (Cincinatti, Ohio November 1992), The Ontario Ministry of Environment Technology Transfer Workshop (November 1992) and the Technical Meetings of the Canadian Pulp & Paper Association (Montreal, January 1993). In addition, they have been discussed with several other organizations, including research groups in Germany, Russia, Finland and Sweden. The data are currently in the final stages of preparation for a submission to a refereed scientific journal (Robinson *et al.*, MS, Munkittrick *et al.*, MS; Servos *et al.*, MS; van den Heuvel *et al.*, MS). A summary is included here (Table 1), but details are unavailable until the peer review process is complete.

This study included bleached kraft mills with and without secondary treatment and three sulphite mills with primary treatment; all mills were located in Ontario (Robinson *et al.*, MS). Increased MFO activities were observed in male white sucker collected at all downstream sites where effluent dilution was not extensive, regardless of pulping process or effluent treatment (Munkittrick *et al.*, MS). Female reproductive organs were significantly smaller at all sites where sufficient numbers of fish were caught, with the exception of one mill, and plasma steroid concentrations were reduced in at least one sex at all sites except one mill (Table 1). Changes in liver size and condition factor were inconsistent, but were present at sites with and without chlorine bleaching. There were no differences in fish responses between kraft mills with secondary treatment, a kraft mill producing primarily unbleached pulp and mills using the sulphite process. However, the mill producing primarily unbleached kraft pulp and the sulphite mills had lower levels of contamination with dioxins and furans than bleached kraft mills (Servos *et al.*, MS). The extent of physiological disruption could not be linked to levels of chlorinated dioxins and furans near the pulp mills since a) dioxin levels were not correlated with effects among fish within sites, and b) high induction was seen at a mill producing primarily unbleached kraft pulp, that had low levels of dioxin. Previous experiments have shown that induction in wild, caged and laboratory-exposed fish disappears within 8 days of transfer to clean water (Munkittrick *et al.*, 1992a, Munkittrick and Servos, unpubl. data), whereas laboratory experiments with dioxins and furans have shown the half-life of induction to be much longer (Muir *et al.*, 1990). MFO induction after laboratory exposure to effluent persists in samples after effluent centrifugation and filtration, as well as secondary treatment (Hewitt and Carey, unpubl. data).

Although receiving water bioassays using both fathead minnow and *Ceriodaphnia* detected toxicity near several mills, there was no correlation of test results with the presence or absence of effects in wild fish, or with levels of AOX in the receiving water or with levels of AOX discharged per tonne of product (Table 1; Robinson *et al.*, MS). This lack of correlation between AOX and toxicity has been reported by other authors, including Borton *et al.*, (1990), Hall *et al.*, (1989), Gergov *et al.*, (1988) and O'Connor *et al.*, (1993). There was no correlation between reproductive effects in wild fish and pulping process, dioxin contamination, AOX production, AOX levels in receiving water or the use of chlorine. MFO activity in male fish showed a very high correlation with effluent concentration, and consequently with AOX and other effluent-related chemicals in receiving water, but not AOX on a kg per tonne production basis.

Another recent approach involves laboratory bioassays of pulp mill effluents to test for their ability to increase liver MFO activities in exposed fish. In fish exposed to effluents from a variety of mill types, Martel *et al.*, (1993) demonstrated that ClO_2 substitution for molecular chlorine as a bleaching agent was not associated with any change in MFO induction in fish, and they proposed that the chemicals associated with the physiological alterations may originate in the pulping process prior to the bleaching stage. Williams (Williams and Carey, unpubl. data) has also employed this assay to test final effluent from kraft mills employing a variety of bleaching processes, including an unbleached kraft mill. All kraft mill effluents tested caused increases in liver MFO activities, including effluent from the unbleached kraft mill.

The presence of effects at mills without chlorine bleaching is also supported by recent field studies from Finland. Increased MFO activity was measured in rainbow trout liver cells exposed to extracts from an unbleached sulfite mill, as well as in wild perch caught in receiving waters near the mill and rainbow trout and whitefish caged in receiving waters near the mill discharge (Lindstrom-Seppa *et al.*, 1992). The extent of induction was comparable to that observed in previous studies of effluent from bleached pulp mills.

Pesonen and Andersson (1992) compared effects on rainbow trout liver cells of diethyl ether extracts of final effluents from five mills (three bleached kraft mills, one unbleached kraft mill and one unbleached sulfite mill). When cellular glutathione was measured as an indicator of oxidative stress, extracts from the unbleached mills were more potent than those from bleached mills at causing depletion of reduced glutathione. In addition, extracts from all five mills markedly increased MFO activity at concentrations well below those affecting the glutathione system. The authors concluded that bioactive substances were present in effluents from all mills, not just those employing chlorine bleaching.

SUMMARY

The physiological disruptions in fish near pulp mills are associated with significant effects on their reproductive development. Canadian studies have clearly shown physiological effects in fish when they are exposed to effluents, whether from mills using chlorine bleaching or not. These effects occur in parallel with increased MFO activity, an enzyme that is involved in the excretion of foreign compounds. These effects are present at very low effluent concentrations (<1%), are not removed during normal secondary treatment of effluent, and existing effluent toxicity tests can not predict whether or not they will be seen in wild fish. These effects do not correlate with AOX or levels of dioxins and furans, and represent a previously unidentified category of impact, distinct from the issue of chlorine use and the potential discharge of persistent, bioaccumulating toxic substances. However, the effects are transient and reversible, and once appropriate changes in process or effluent treatment have been designed, recovery of wild fish populations should happen quickly.

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Table 1. Summary of effects recorded during the survey of receiving water effects. Data are summarized for 7 d receiving water bioassays using fathead minnow (FHM) and Ceriodaphnia, and physiological responses recorded in wild white sucker collected near the discharge (This Table has been modified from Munkittrick and Van Der Kraak 1993; + increased, - decreased, 0 no change, * results different from only 1 reference site). The actual data are reported in detailed manuscripts currently under review. Bioassay data are recorded in Robinson et al., MS; physiological responses in Munkittrick et al., MS and dioxins analyses and comparisons in Servos et al., MS and van den Heuvel et al., MS.

Mill	Process	Effluent	Fathead minnow		Ceriodaphnia		Males					Females				
		Treatment	Survival	Growth	Survival	Reproduction	MFO	Steroid	GSI	LSI	TCDD TEQs[b]	MFO	Steroid	GSI	LSI	TCDD TEQs[b]
A	Kraft	Secondary	0	0	0	+	+	-	-	+	+++	+	-	-	0	+++
B	Kraft	Secondary	0	0	0	+	+	-	0	0	++	+	0	0	0	++
C	Kraft	Secondary	0	0	0	0	+	-	0	+	++	+	-	-	0	+
D	Kraft	Secondary	0	0	0	+	NC[c]									
E	Kraft	Primary	-	0	0	+	+	-	*[d]	*	+	+	*	-	0	+
F	Kraft	Primary	0	0	0	-	+	-	0	0	+++	+	-	-	0	+++
G[a]	Kraft	Primary	-	0	0	+	+				++	+				NC
H[a]	Kraft	Primary	0	0	0	0	+[e]				+++	+				+++
I[a]	Kraft	Primary					0				NC[c]	0				NC
J	Sulphite	Primary	-	0	0	-	+	0	0	+	+	0	0	-	+	+
K	Sulphite	Primary	-	0	0	0	+	0	-	+	+	0	-	-	+	+
L	Sulphite	Primary	0	0	0	+	NC[c]									

[a] insufficient numbers of fish for additional analysis
[b] liver levels as measured by rat H4IIE assay (+ <10 ppt, ++ <40 ppt, +++ >40 ppt)
[c] not conducted at this site
[d] interpretation not consistent with both reference sites
[e] longnose sucker

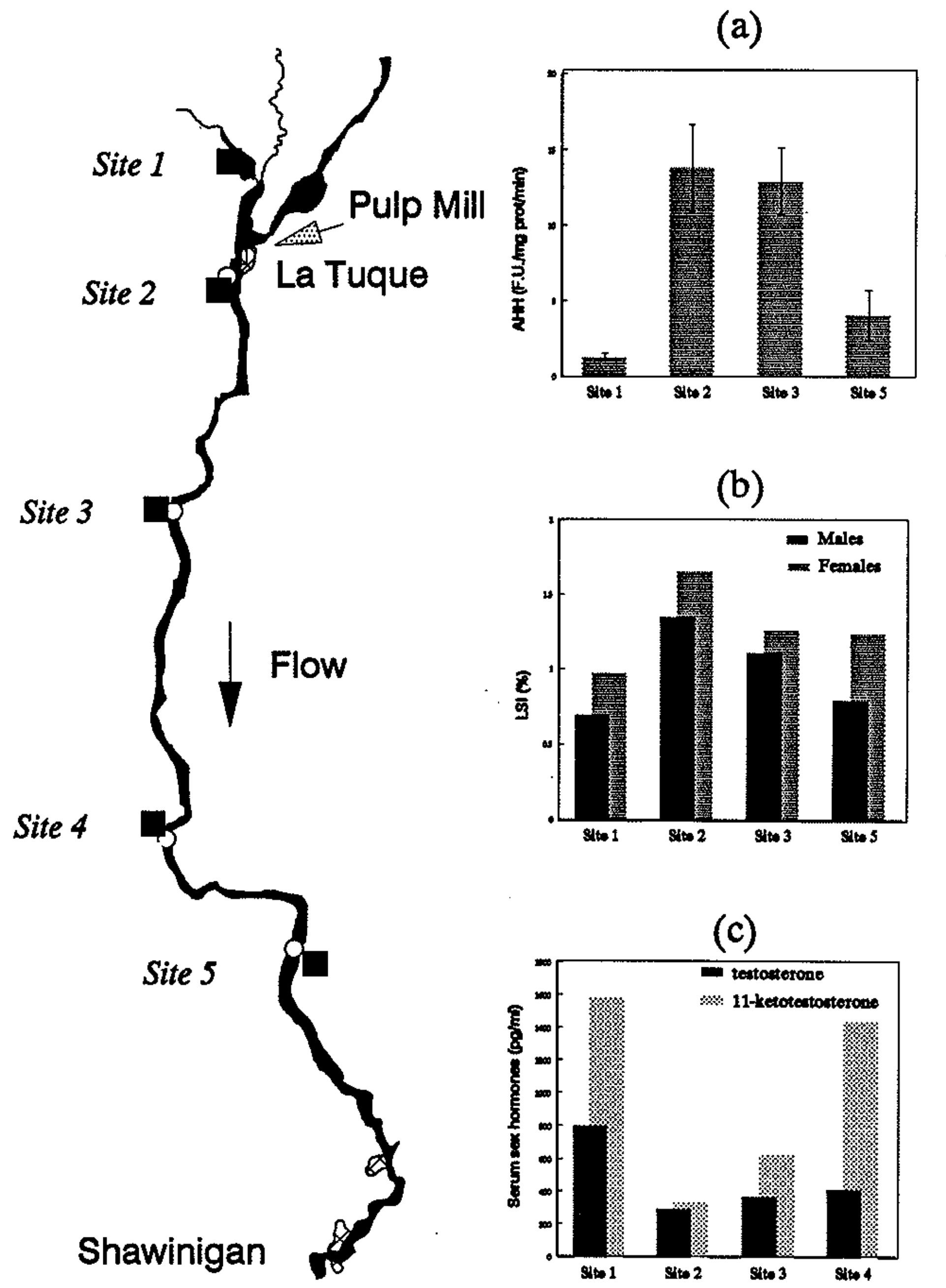

Figure 1. Sampling site locations and biochemical responses in white sucker from the St. Maurice River, 1989: (a) liver MFO activity; (b) liver somatic index; (c) male sex hormones. Relative to the mill discharge, site 1 is 10 km upstream while sites 2, 3 and 5 are 2, 32 and 95 km downstream. (Hodson *et al.*, 1992).

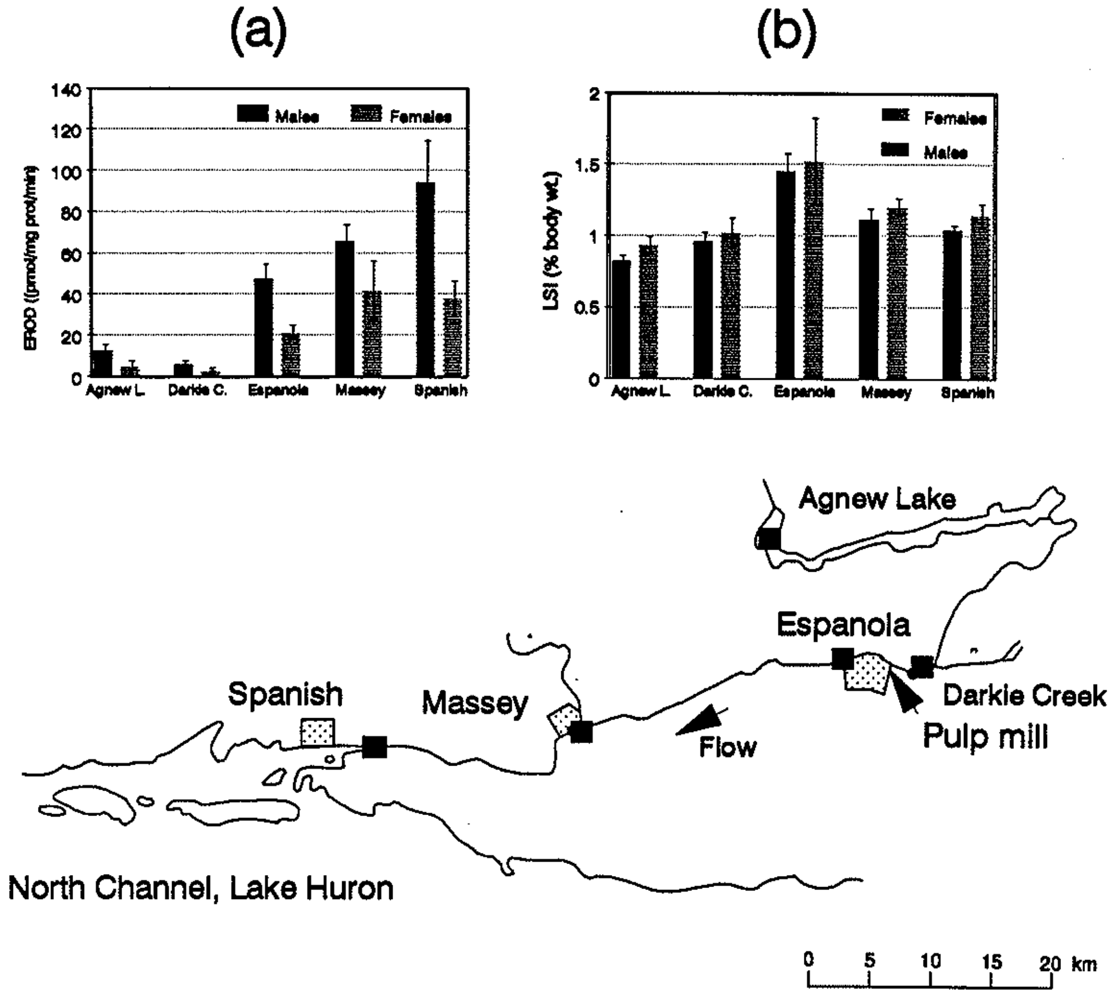

Figure 2. Sampling sites and biochemical responses in wild white sucker from the Spanish River, 1990: (a) liver MFO activity; (b) liver somatic index. (Servos *et al.*, in press).

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