# Seasonal Effects of Bleached Kraft Mill Effluent on Reproductive Parameters of White Sucker (*Catostomus commersoni*) Populations of the St. Maurice River, Quebec, Canada[1]

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Reproductive parameters and accumulation of chlorophenolic compounds by white sucker (*Catostomus commersoni*) populations exposed to bleached kraft mill effluent (BKME) were studied in the St. Maurice River, Quebec. Compared with unexposed populations from the same waterway, exposed white sucker showed obvious effects of chemical exposure as far as 100 km downstream of the mill, as revealed by a strong induction of ethoxyresorufin *O*-deethylase (EROD) activity. In males, circulating plasma testosterone levels were the same at all sites, while 11-ketotestosterone levels were significantly lower at exposed stations. In females, testosterone and 17β-estradiol levels were significantly reduced at the two exposed stations relative to the reference station. Despite different hormone levels during sexual maturation, gonad weight as a proportion of carcass weight was similar at all sites for both sexes during early gonadal development. The relationship between carcass weight and fecundity was more variable at the station immediately downstream of the mill. Biochemical and physiological parameters measured in this study do not allow us to clearly relate perturbations in plasma steroid levels to impaired reproduction as measured by gonad weight and fecundity.

Des populations de meunier noirs (*Catostomus commersoni*) de la rivière St-Maurice, Québec, exposées aux effluents d'une usine de pâte kraft blanchie ont été étudiées. Divers aspects de la reproduction ainsi que l'accumulation de composés chlorophénolés par ces poissons furent évalués. Comparés à une population non-exposée provenant de la même rivière, les meuniers noirs capturés aussi loin qu'à 100 km en aval de l'usine démontrent des effets évidents d'une exposition chimique tel que révélé par une activité très élevée de l'ethoxyresorufine *O*-dééthylase (EROD). Chez les poissons mâles, les niveaux de testostérone plasmatique ne variaient pas entre les sites, alors que les niveaux de 11-kétotestostérone étaient significativement plus bas chez les meuniers exposés. Chez les femelles, la testosterone et le 17β-estradiol étaient significativement plus bas chez les populations exposées. Malgré des niveaux hormonaux réduits, le développement des gonades en proportion du poids de la carcasse était similaire durant l'automne. La relation entre le poids de la carcasse et la fécondité était beaucoup plus variable à la station située immédiatement en aval de l'usine. Les paramètres biochimiques et physiologiques mesurés lors de cette étude ne permettent pas de relier les perturbations des hormones stériodes plasmatiques à des troubles de reproduction tel que mesuré par le poids des gonades et la fécondité.

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Recent research in Scandinavia and Canada has demonstrated various impacts of bleached kraft mill effluent (BKME) on fish. Among others, induction of mixed function oxygenase enzymes (MFO) was observed (Andersson et al. 1988; Lindström-Seppä and Oikari 1988). In fish, the oxidative transformation of xenobiotics can be carried out by the cytochrome P-450 family of enzymes or MFOs (Stegeman and Kloepper-Sams 1987). The cytochrome P-450 system

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TABLE 1. Various responses observed in investigations of impacts of BKME on fish. + = increase, 0 = no change, and − = negative effect of BKME exposure on fish relative to reference populations.

| | Fourhorn sculpin (*Myoxocephaelus quadricornis*) Andersson et al. 1987 | European pearch (*Perca fluviatilis*) Sandström et al. 1988 | European pearch (*Perca fluviatilis*) Karas et al. 1991 | White sucker Munkittrick et al. 1991 | Redbreast sunfish (*Lepomis auritus*) Adams et al. 1992 |
|---|---|---|---|---|---|
| Length at age | − | + | −[a] | − | + |
| Weight at age | − | | | − | |
| Growth rate | | + | | − | 0 |
| Lipid accumulation | | +[b] | | + | + |
| Gonadosomatic index | | − | | − | |
| Gonad weight versus carcass weight | | − | | − | |
| Age at maturity | | | + | + | |
| Fecundity | | | +[c] | −[d] | 0 |
| Steroid hormones[e] | | | | − (T), 0 (E) | − (E) |
| MFO induction | + | | | + | + |

[a]Length of embryos.
[b]Reported by condition factor.
[c]Measured by the total number of roe strings on artificial substrate.
[d]Young females only.
[e]T, testosterone in males; E, 17β-estradiol in females.

plays a central role in detoxification of xenobiotics as well as in the transformation of endogenous substances such as steroid hormones (Lehtinen 1990). Although no direct links have been demonstrated between MFOs and steroid hormones, it had been suggested that induction of MFOs by environmental contaminants could have a severe impact on the biotransformation of steroid hormones in fish and on the reproductive success of exposed populations (Lee 1988). Other effects, such as altered immune responses (Andersson et al. 1988; Larsson et al. 1988), differential growth, and impaired reproduction (Larsson et al. 1988), have also been observed. Because similar chlorine bleaching processes are used in North American pulp mills, various studies have been undertaken in the United States and Canada to establish and describe the impacts of BKME on indigenous fish populations.

Various North American studies describe effects similar to those observed in Scandinavia. Although MFO induction seems consistent in BKME-exposed fish, many responses at the physiological and population level are not consistent and seem to be site specific (Table 1). Investigations of white sucker (*Catostomus commersoni*) conducted by McMaster et al. (1991) and Munkittrick et al. (1991) in Jackfish Bay, Lake Superior, indicated reduced levels of virtually all plasma sex steroids for both sexes, along with reduced gonad size and high MFO activity. A preliminary study of the same species conducted by Hodson et al. (1992) on the St. Maurice River, Quebec, also showed decreased serum testosterone in males, but an increase of this hormone in females, although variations were not statistically significant. Hodson et al. (1992) showed a reduced gonad size for both sexes and an MFO activity related to distance from the mill. Our present extensive survey on the St. Maurice River was undertaken to investigate in detail the effects of BKME exposure on fish populations.

A major bleached kraft pulp and paper mill located at La Tuque, Quebec, on the St. Maurice River produces about 1400 tonnes per day of air-dried product and discharges about 8–10 kg of chlorinated compounds per tonne of product, equivalent to more than 10 tonnes per day of chlorinated chemicals (Hodson et al. 1992). Before being discharged to the river, the effluent receives a primary treatment through a settling basin. No other industries or towns occur along the river until Grand-Mère, Quebec, more than 100 km downstream of La Tuque (Fig. 1). Hydroelectric dams present at 0 and 100 km downstream from the mill control log floating and limit fish movements upstream and downstream of the mill. Thus, single industry contamination combined with limited fish movements provides a unique opportunity to study the effects of BKME on fish populations.

Our major objective was to establish whether a correlation exists between the induction of the MFO system and reproductive impairments coincident with decreased steroid levels by comparing one unexposed population of white sucker located upstream of the mill with two BKME-exposed populations located downstream of the mill.

## Methods and Materials

### Methodological Approach

White sucker is one of the most studied species in environmental investigations of pulp mills in North America. It is common throughout rivers and lakes of Canada and the northern United States, but is not heavily exploited by either sport or commercial fisheries and is consequently not affected by overfishing. Because they are bottom feeders, white sucker are directly exposed to contaminated sediments and are thus likely to exhibit responses to environmental toxicants. Their well-documented biology and the number of other studies of their response to BKME make the white sucker an ideal species for this study (Munkittrick and Dixon 1989).

Evidence of exposure to BKME among the three groups of white sucker was assessed by analysing tissue samples for the presence and concentrations of chlorophenols and chloroguaiacols, compounds typical of BKME. To ensure

that environmental contamination was due mainly to BKME, tissue levels of polychlorinated biphenyls (PCBs) and several organochlorine insecticides not usually found in BKME were also determined.

Induction of the liver MFOs as measured by ethoxyresorufin *O*-deethylase (EROD) was evaluated and related to chemical exposure. Circulating plasma levels of testosterone and 11-ketotestosterone in males and 17α,m20β-dihydroxy-4-pregnen-3-one (17α,20β-diOHprog), testosterone, and 17β-estradiol in females were monitored to evaluate the possible interaction of MFOs and steroid hormones at various stages of reproductive development. Sexual maturation was monitored by measuring gonad weight in relation to body weight of males and females and fecundity of females. We expected the downstream groups of white sucker to exhibit elevated levels of chlorophenolic contamination, greater induction of EROD activity, lower levels of steroid hormones, and retarded gonad development relative to white sucker sampled upstream of the mill.

## Sampling Sites

White sucker were sampled with gill nets in the St. Maurice River, Quebec (Fig. 1) during May 8–20 (spawning), August 1–10, and November 13–22, 1990, and May 6–17, 1991 (spawning). At each sampling period, fish were collected at three sites. The reference station 1 (5 km upstream of the mill) is isolated from the downstream stations by a hydroelectric dam. In this part of the river, current is slow and the bottom is covered with bark from floating logs. Station 2, located immediately downstream of the mill, had a faster current and a bottom that was a mixture of bark and sand. Station 3, 95 km downstream of the mill, resembled station 1. There was continuous log floating at all three stations. At each sampling period, temperature was recorded daily and did not differ from one site to another.

At all three stations, the nets were set in 2–5 m of water, in areas with a relatively slow current. Gill nets were composed of three 50-m panels of 8.9-, 11.4-, and 12.7-cm stretched mesh. Fish were removed from the nets at least once a day and kept alive in an aerated tank on the boat. Fish were transported to the land-based laboratory within 30 min for sample collection.

## Sample Collection

For the first 30 fish sampled alive at each station, a blood sample was taken by caudal puncture, allowed to clot on ice for 15 min, centrifuged, and the serum quick-frozen in liquid nitrogen for subsequent hormone analysis. The liver was removed, rinsed with an ice-cold solution of 0.15 M KCl, cut into small pieces, and frozen in liquid nitrogen. The length of each fish and weights of the whole body, gonads, liver, intestines, and carcass (whole body minus viscera) were recorded. The first pectoral fin rays were kept to age the fish. Each carcass was immediately frozen on dry ice for contaminant analysis. In the spring, ovaries were immersed in Gilson's solution (Bagenal 1978) for evaluation of fecundity. Fish that had ovulated eggs in the body cavity were not used to evaluate fecundity or steroid levels.

## Sample Analyses

Fish carcasses were kept frozen at −20°C until homogenization. The carcass was passed through a Hobart meat grinder three times to ensure a homogenous mix and the homogenate was frozen. The grinder was thoroughly cleaned with soap and rinsed with water and acetone after homogenization of each fish.

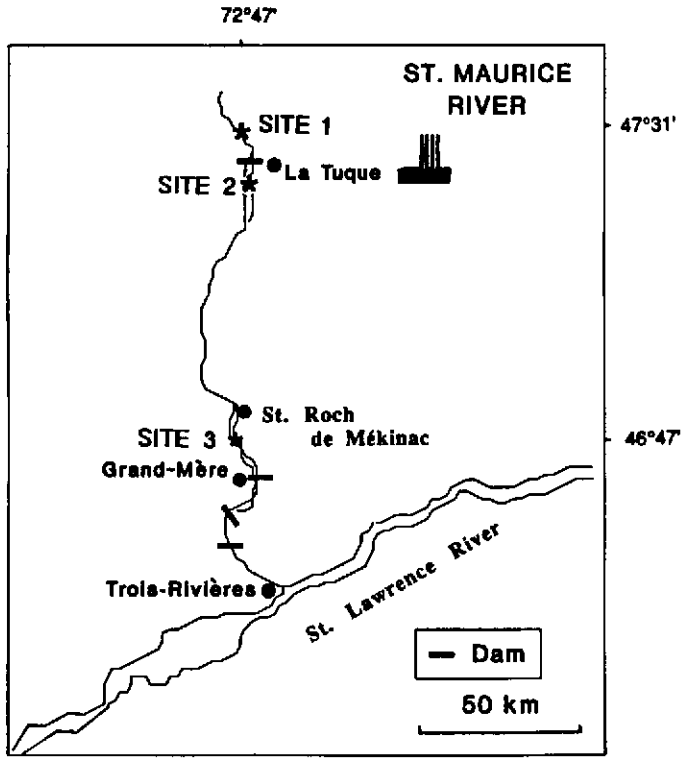



FIG. 1. St. Maurice River and stations sampled in 1990 and 1991. A pulp mill is located at La Tuque and hydroelectric dams at La Tuque and Grand-Mère. Site 1 is 10 km upstream from the pulp mill; sites 2 and 3 are 2 and 95 km downstream of the mill, respectively.

Carcasses of 10 males and 10 females from station 3 were analysed for concentrations of chlorophenolic compounds to test for possible differences of contamination between sexes. No significant differences were found in contamination levels between sexes: for example, 4,5,6-TCG was 1.52 ± 0.51 ng·g wet weight$^{-1}$ for males and 0.99 ± 0.33 ng·g$^{-1}$ for females. Therefore, sexes were pooled for all other analyses. For subsequent analyses, two subsamples of homogenates of 5 g each originating from two individuals of a similar origin, age, and length were pooled together to better estimate the average contamination of the different fish populations. Fish that could not be paired in this fashion were analysed individually. Although pooling samples prevents evaluating individual variability in contamination, our only intent was to discriminate between exposed and unexposed populations according to the concentration of phenolics. Extraction, cleanup, and gas chromatography methods were carried out according to Carey et al. (1988). Analyses of PCB isomers and organochlorine insecticides followed the method of Comba et al. (1989), using a pool of 10 fish for each station. The detection limit of the gas chromatograph was 0.1 ng·g wet weight tissue$^{-1}$, the percent recoveries of standards varied between 80 and 100%, and the variability of analysis for samples was <12% (six samples compared). Contamination data reported here were not corrected for percent recovery.

TABLE 2. Organochlorine pesticides and ΣPCBs (ng·g wet weight$^{-1}$) in white sucker from the St. Maurice River. ΣPCBs represents the summation of 137 isomers monitored; $n$ = 10 per station; — = not detected.

| | Station 1 | Station 2 | Station 3 |
|---|---|---|---|
| HCB | 0.7 | 1.4 | 0.7 |
| Heptachlor | — | — | — |
| Aldrin | — | 0.9 | 0.9 |
| *o,p*-DDE | — | — | — |
| *p,p*-DDE | 2.9 | 2.6 | 1.9 |
| *o,p*-DDT | 0.3 | 0.4 | — |
| *p,p*-DDT | — | — | — |
| *o,p*-DDD | 3.3 | 3.0 | 1.1 |
| *p,p*-DDD | 3.5 | 5.0 | 2.0 |
| Mirex | 0.4 | 0.9 | 0.2 |
| α-BHC | 0.8 | 1.5 | 0.9 |
| β-BHC | 0.1 | — | — |
| δ-BHC | 0.6 | 0.7 | 0.4 |
| Heptachlor epoxide | 1.9 | 1.8 | 0.9 |
| δ-Chlordane | 0.8 | 0.8 | 0.4 |
| α-Endosulfan | 1.1 | 0.9 | 0.5 |
| α-Chlordane | 1.5 | 4.5 | 2.5 |
| Dieldrin | 3.0 | 4.0 | 2.0 |
| Endrin | 1.3 | 2.0 | 0.6 |
| β-Endosulfan | 2.1 | 2.0 | 0.8 |
| Methoxychlor | 5.5 | 6.0 | 5.9 |
| ΣPCBs | 4.7 | 5.3 | 4.0 |

Liver samples were kept at −80°C until analysed for enzymatic activities, which was done within 2 wk of sampling. In the laboratory, samples were thawed on ice and analysed for EROD activity in the S-9 fraction of the tissue homogenate, following the spectrofluorometric method described in Hodson et al. (1991).

Blood samples from the caudal artery were collected in May, August, and November 1990 and kept at −80°C until analysed. Following ether extraction of the plasma, 17α,20β-diOHprog, testosterone, 17β-estradiol, and 11-KT were measured by radioimmunoassay (RIA). A description of the antisera used to measure 17α,20β-diOHprog, testosterone, and 17β-estradiol is given in Van Der Kraak et al. (1984), and the 11-KT antisera is described in Wade and Van Der Kraak (1991). All samples were assayed in duplicates and the interassay variability was less than 8%.

Gonads were soaked in Gilson's solution for 2 wk to ensure hardening of eggs and dissolution of ovarian tissue. They were subsequently dried in a low-temperature oven for 24 h at 60°C. Three subsamples were counted and weighed to provide an estimate of fecundity for females of different sites. The dry weights of individual eggs, as calculated by the dry weight of gonad divided by the fecundity counts, were compared using an ANOVA.

Because transformation did not normalize the data set, statistical comparisons of contamination levels, EROD, and steroid levels were done using a Kruskal–Wallis test followed by nonparametric multiple comparisons with unequal sample sizes. Regressions of gonad weight to carcass weight and of fecundity to female carcass weight were compared among sites using a covariance analysis with carcass weight as a covariable and station as a classification factor. When ANCOVA revealed parallel slopes, the analysis was pursued by contrasts on adjusted means to detect any differences between stations. When the analysis revealed nonparallel slopes, a posteriori tests were performed to identify which slopes differed from the others. Normality of data sets was verified by a Sharpio–Wilk test and homogeneity of variances was checked by a Bartlett test before applying the covariance analysis (Zar 1984).

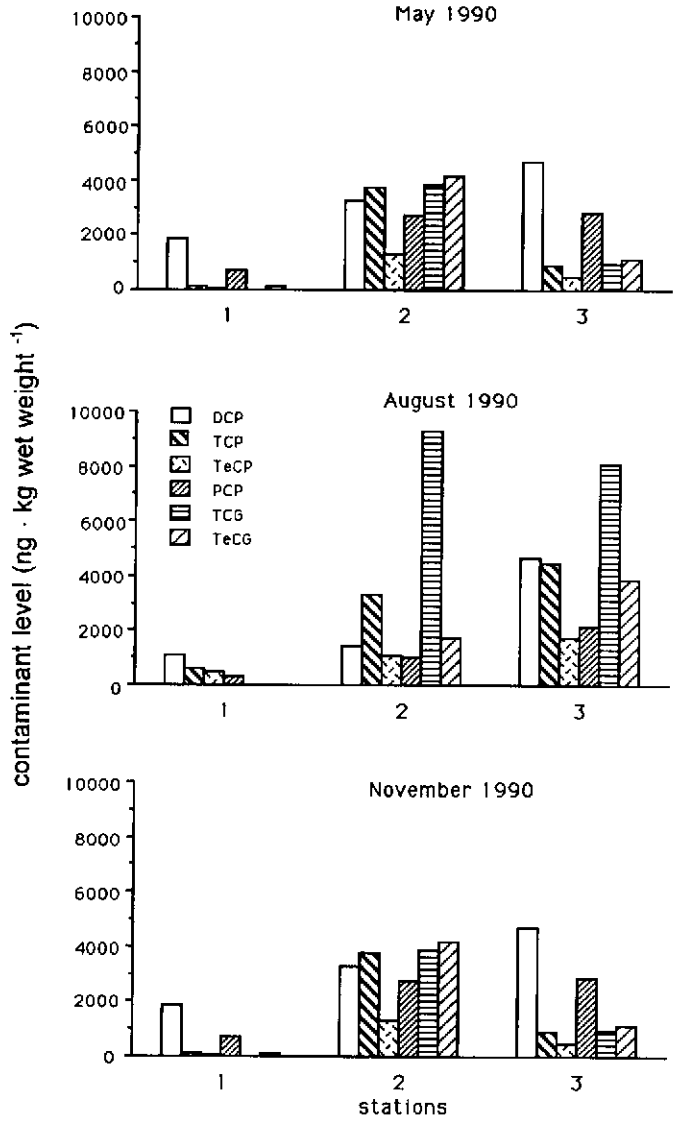



FIG. 2. Chlorophenol and chloroguaiacol levels in white sucker from the St. Maurice River in 1990. For stations 1, 2, and 3, $n$ = 67, 37, and 53, respectively. DCP, dichlorophenols (2,4-DCP, 3,4-DCP); TCP, trichlorophenol (2,4,6-TCP, 2,4,5-TCP, 2,3,4-TCP, 2,3,5-TCP); TeCP, tetrachlorophenol (2,3,4,6-TeCP); PCP, pentachlorophenol (1,2,3,4,5-PCP); TCG, trichloroguaiacol (3,4,5-TCG); TeCG, tetrachloroguaiacol (2,3,4,6-TeCG).

## Results

White sucker from the three stations exhibited minor contamination by organochlorine insecticides (Table 2). Contamination in the carcasses of white sucker from all sites varied from lower than the detectable level (0.1 ng·g wet weight flesh$^{-1}$) to a maximum of 6.0 ng·g wet weight$^{-1}$ for methoxychlor. Some PCB isomers were also present in the fish carcasses, although the levels were very low. Isomer

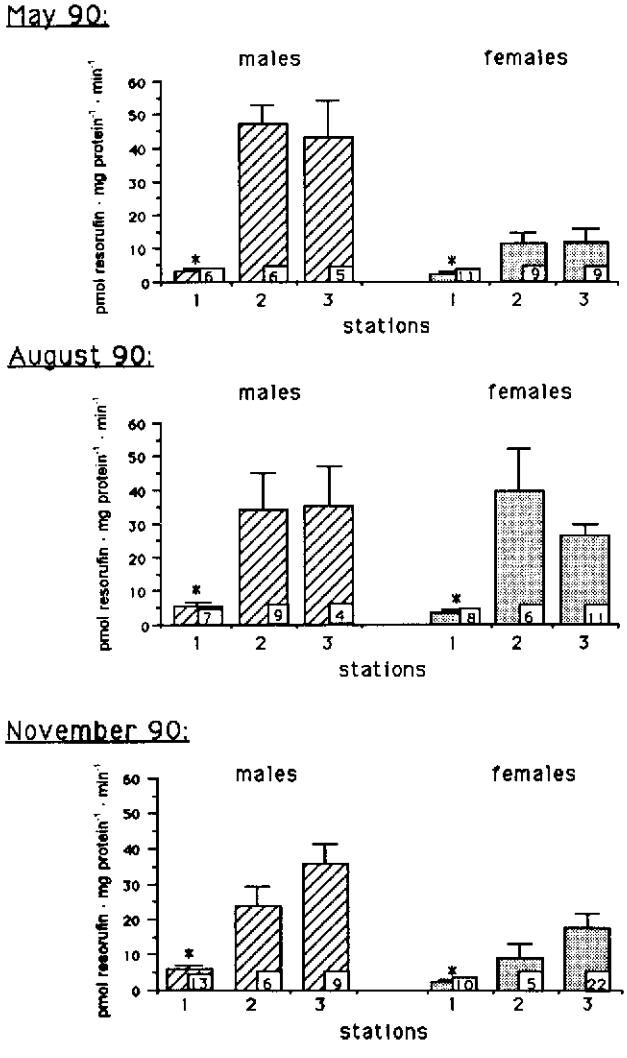



FIG. 3. EROD activity in white sucker sampled in spring, summer, and fall 1990. Error bars represent standard errors. Sample sizes ($n$) are given in the lower right corner of the histograms; *difference ($p < 0.05$) between reference and exposed stations.

153, which is often the most abundant in biota (Ballschmiter et al. 1981), was the dominant PCB isomer, with a maximum recorded level of 0.05 ng·g wet weight$^{-1}$.

Fish sampled downstream of the mill had higher ($p < 0.05$) tissue levels of chlorophenols and chloroguaiacols than fish sampled upstream (Fig. 2). The levels of contamination at stations 2 and 3 were generally similar. Comparisons over time revealed that the low level of contamination at station 1 was similar throughout the year ($0.12 < p < 0.86$). Levels of chlorophenolic compounds at stations 2 and 3 were generally similar throughout the year, with the exception of TeCP, TCG, and TeCG at station 3 which were found at higher levels in August relative to May ($0.03 < p < 0.001$). The high levels of TCG and TeCG at stations 2 and 3 allowed us to conclude that fish populations were exposed to BKME and to associate observed effects with BKME exposure. It was not intended in this study to relate a specific level of contamination in a few fish to specific effects on populations.

There were no relationships among the BKME-related contaminant level (taken individually or as a summation) in the carcass and length, whole-body weight, carcass weight, or age (data not shown).

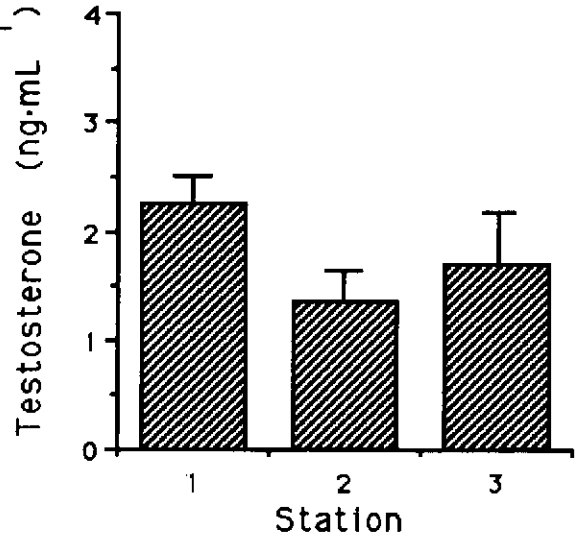
Male and female white sucker sampled downstream of the mill exhibited higher levels of EROD activity ($p < 0.001$) than fish from the reference station (Fig. 3). In August only, enzyme induction in female white sucker followed the same patterns and levels as in males. Among BKME-exposed female white sucker, induction was lowest in May and November, compared with males sampled at all times or with females sampled in August.

Steriod hormone levels of males and females sampled in May 1990 were highly variable, partly due to the spawning activity which causes major and rapid steriod changes during final gonadal maturation (Scott et al. 1984). Gonad development of white sucker sampled on the spawning ground was not perfectly synchronous, which may also contribute to the high variability of sex steroid levels observed in May 1990. In August, both males and females were reproductively inactive (GSI = gonad weight divided by carcass weight <3.0 for both sexes) at all stations resulting in very low levels of hormones. Due to reduced hormone levels in August, no site differences were observed. Because of these confounding factors, data from May and August were unsuitable for comparisons among sites.

In November, white sucker were maturing sexually for spring spawning, and steroid levels were elevated and homogenous in the different populations, permitting valid comparisons among sites. At this time, plasma sex steroid levels were altered in BKME-exposed fish relative to the reference ones. In males, testosterone levels were similar at all sites in November 1990 ($p = 0.150$) (Fig. 4), while 11-ketotestosterone serum levels were reduced at exposed sites ($p = 0.0048$). In females, 17α,20β-diOHprog levels were higher at exposed stations ($p = 0.014$). In contrast with males, female white sucker exposed to BKME exhibited a significant reduction in testosterone ($p = 0.014$) and 17β-estradiol ($p = 0.001$) levels relative to females from the reference site (Fig. 5). For both sexes, no significant correlations between MFO induction and any steroid levels were found ($p < 0.05$).

Male white sucker sampled in November showed no differences among sites in the linear regressions relating gonad and carcass weight ($p = 0.95$, intercept: $p = 0.61$; data not shown). In the following spring, however, male white sucker from different stations had different gonad to carcass weight relationships ($p = 0.0002$) (Fig. 6), with the slope of site 1 being different than the slope of site 2 ($p = 0.013$). The slope at site 3 was not statistically different from that observed at sites 1 and 2. Given the fact that slopes were significantly different and $r^2$ values were low, statistical comparisons of means were not pursued.

Despite lower levels of hormones at downstream stations in November 1990, all females exhibited a similar gonad weight for a given carcass weight (slopes: $p = 0.93$, intercept: $p = 0.59$; data not shown). In May 1991, females still exhibited comparable gains in gonad weight relative to carcass weight (i.e., parallel curves with $p = 0.15$) (Fig. 7A). However, differences were observed in the elevations of the curves (i.e., the $y$-intercept was different, $p = 0.002$), which indicates that, on average, some female white sucker had a larger gonad weight for a given carcass weight. A posteriori tests on adjusted means revealed that reference site 1 was different from the exposed site 3 ($p = 0.0005$), with fish from



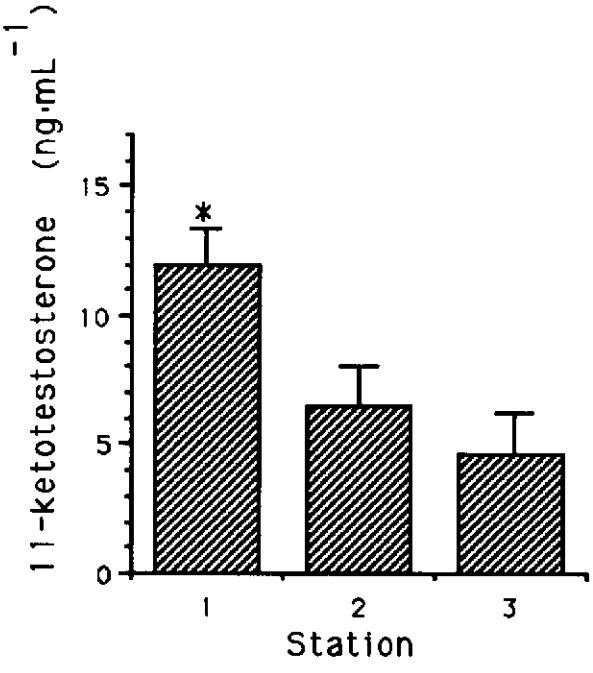




FIG. 4. Testosterone and 11-ketotestosterone blood serum levels in male white sucker from the St. Maurice River in November 1990; $n$ = 5, 6, and 8 for stations 1, 2, and 3, respectively. Error bars represent standard errors; *difference ($p < 0.05$) between reference and exposed stations.

site 1 having a higher gonad weight for a given carcass weight. Females from exposed site 2 were intermediate between sites 1 and 3, and therefore did not differ from other sites ($p > 0.14$) in gonadal development (Fig. 7B).

Significant carcass weight related fecundity differences were observed among the three stations the following spring (May 1991) just prior to spawning (Fig. 8). A multiple comparison procedure showed that reference site 1 and contaminated site 3 were different from contaminated site 2 ($p = 0.001$). Females at site 2 showed the greatest variability in the weight–fecundity relationship with carcass weight explaining only 22% of the variation in fecundity. The dry weight of individual eggs, as calculated by the dry weight of gonad divided by the fecundity counts, was not different among stations ($p = 0.17$).

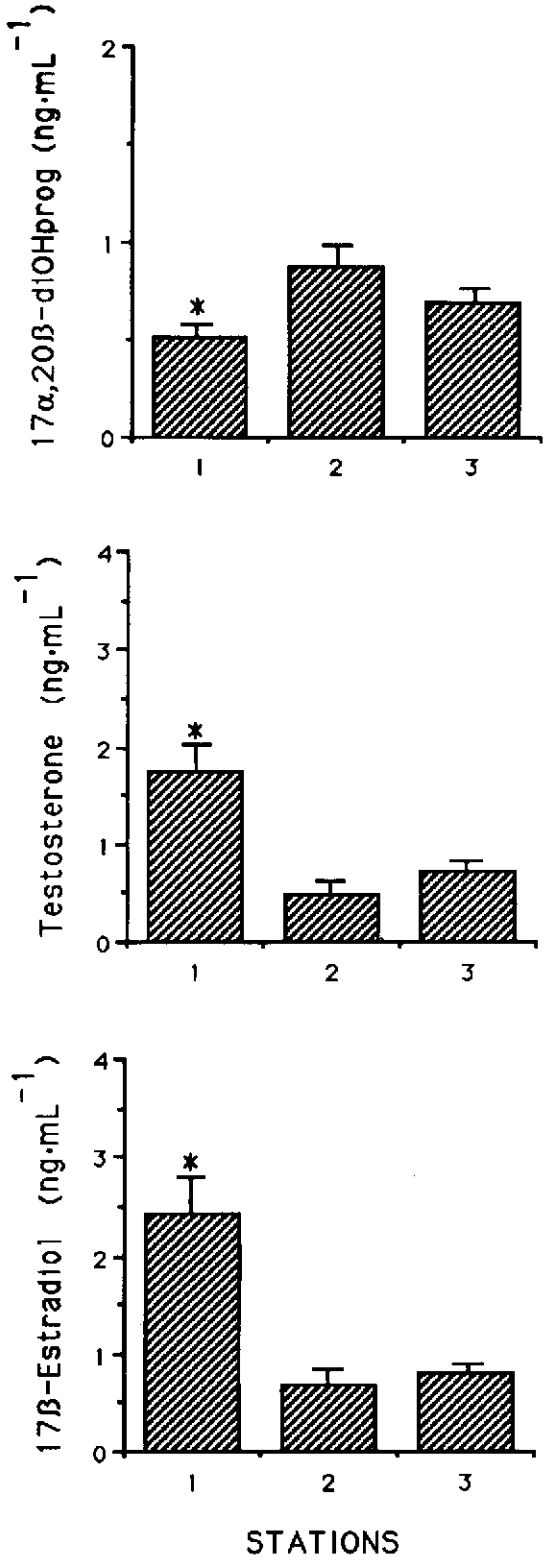



FIG. 5. 17α,20β-diOHprog, testosterone, and 17β-estradiol blood serum levels in female white sucker in November 1990; $n$ = 16, 6, and 23 for stations 1, 2, and 3, respectively. *difference ($p < 0.05$) between reference and exposed stations.

## Discussion

Contamination of white sucker populations from the St. Maurice River by organochlorine insecticides may be

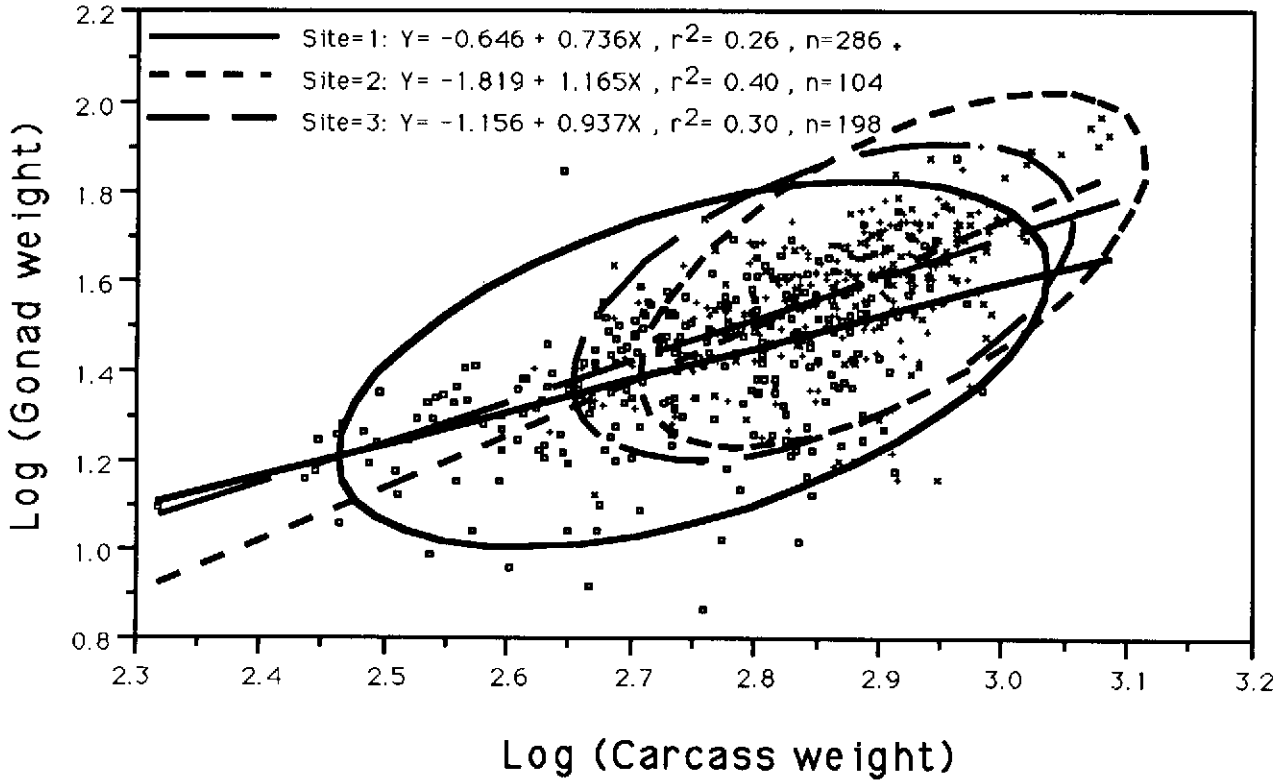



FIG. 6. For male white sucker sampled in May 1991, regressions between the logarithm of gonad weight to the logarithm of carcass weight indicate differences between sites 1 and 2 in the gain of gonad weight relative to a gain in carcass weight ($F = 3.67$, $p = 0.0259$). □, reference site 1; ×, site 2; +, site 3. Ellipses include 95% of data points at each site.

considered negligible when compared with fish from other locations. Trépanier (1984) described PCB concentrations in white sucker originating from other major tributaries of the St. Lawrence River; PCB concentrations were rarely lower than 1000 ng·g wet weight$^{-1}$. In our study, the low contamination by organochlorine insecticides was similar at all locations, indicating a low level diffuse or point source upstream of the reference station. It is not known if such background contamination would have any biological effects on fish on a long-term basis. Considering that organochlorine compounds such as DDT metabolites and PCB isomers are bioaccumulated rather than metabolized and/or excreted (Paasivirta et al. 1985), low levels of those compounds indicate a chronic, low-level exposure. There is no long-term laboratory study known to us that describe adverse effects related to such a low level of contamination. We therefore considered that biological effects observed in white sucker from the St. Maurice River were mainly due to BKME exposure.

Chlorophenols and chloroguaiacols, indicators of BKME contamination, were found in white sucker from all sites. Levels observed at the reference site were much lower than at downstream sites. Despite their biodegradability, chlorophenolic compounds originating from chlorine bleaching processes have some accumulation potential (Paasivirta et al. 1985). Dichlorophenols were the most abundant phenolic compounds found at the upstream station. Other studies on chlorophenolic compounds in areas free from pulp and paper mills indicate that some chlorophenols may originate from air transport (Paasivirta et al. 1985). This may be the case for dichlorophenols because station 1 is located 10 km upstream of the pulp mill. Fish from stations 2 and 3 are similar in their contamination levels, suggesting that individuals inhabiting those two stations are exposed to similar BKME concentrations with little dilution downstream.

In May 1990, MFO activity among females collected at exposed sites was statistically greater than that of females collected at the reference site. However, in females, reduced MFO levels in May relative to August may be related to the spring spawning activity, when steroid hormones can depress MFO levels. Stegeman et al. (1982) showed that administration of 17β-estradiol in immature brook trout (*Salvelinus fontinalis*) depressed levels of hepatic cytochrome P-450. Pajor et al. (1990) also showed that 17β-estradiol causes a strong suppression of microsomal P-450 content in brook trout. The presence of 17β-estradiol may thus be responsible for MFO inhibition or inhibition of MFO induction in females. 17β-Estradiol could act through a negative feedback mechanism, but other mechanisms contributing to sex differences in MFO activity could also intervene. In August, hormone levels were lowest relative to May and November, and EROD activities in both sexes were similar. In November, white sucker were undergoing gonad maturation for the spring spawning. Steroid levels were already high and there was a corresponding depression of EROD activity in females. At present, however, a direct link between EROD and steroid hormones has not been demonstrated either in vitro or in vivo.

White sucker collected downstream of the mill showed a clear response to chemical exposure as indicated by a strong induction of the liver enzyme EROD. Induction is one of the most consistent responses to BKME exposure and may indicate the presence of polycyclic aromatic compounds such as chlorinated dibenzo-*p*-dioxins (Hodson et al. 1991).

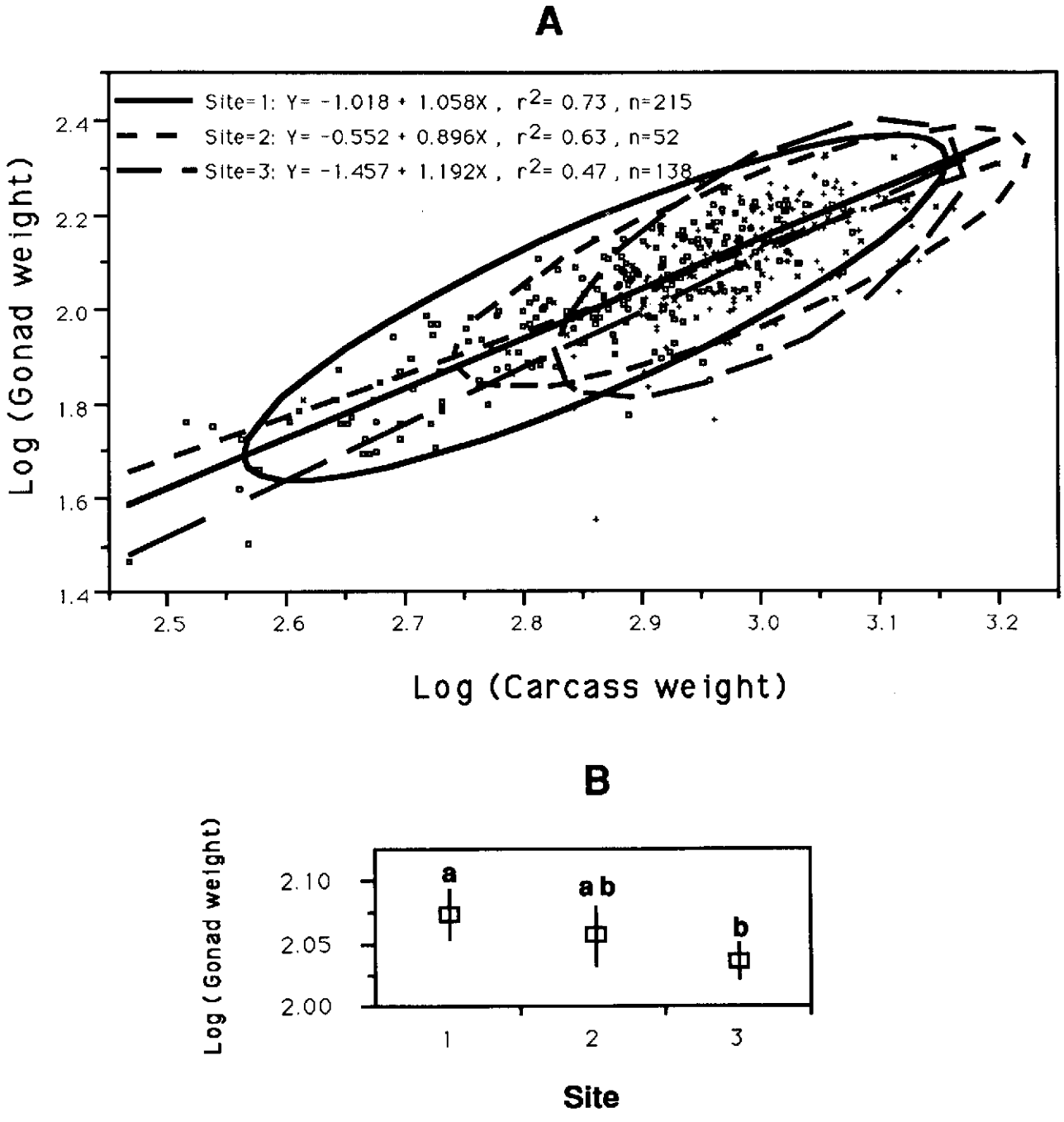



FIG. 7. (A) For female white sucker sampled in May 1991, all slopes are parallel ($F = 1.92$, $p = 0.15$). □, reference site 1; ×, site 2; +, site 3. Ellipses include 95% of data points at each site. (B) Multiple comparisons of adjusted means of the three sites indicate that all stations are not equal. Different letters designate a statistical difference ($p < 0.05$). Error bars represent the confidence interval at 95%.

Dioxins and furans, which have been shown to be strong MFO inducers (Niimi and Oliver 1989), were also detected in carcasses of white sucker from the St. Maurice River in 1989 (arithmetic averages over the three sites of 13.1 pg tetrachlorodioxins·g$^{-1}$ and 107 pg tetrachlorodibenzofurans·g$^{-1}$, 11 fish pooled, Hodson et al. 1992) and may be partially responsible for MFO induction. Due to the nature of BKME effluent, which contains over 300 components, there may be other products or combinations of products that could act as inducers of cytochrome P-450.

In fish exposed to pulp mill effluents, lower titers of steroid hormones are often observed in conjunction with induced MFO. In this study, similar levels of testosterone were observed in males from all stations in November 1990, suggesting that exposure to BKME did not influence plasma concentrations of this hormone. However, levels of 11-ketotestosterone were lower at BKME-exposed stations. Because 11-ketotestosterone is a metabolite of testosterone in fish (Ozon 1972), lower levels of 11-ketotestosterone suggest a disruption in the male steroid biosynthetic pathway somewhere before 11-ketotestosterone is synthesized (Fig. 4). Impairment could occur at multiple sites, possibly at the

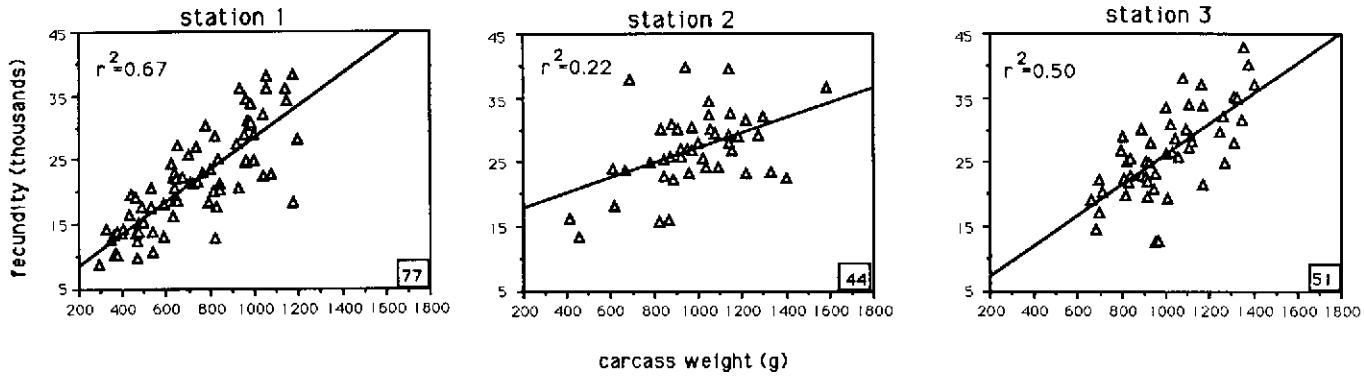



FIG. 8. Fecundity versus carcass weight of females sampled in May 1991. Fecundity varies differently with carcass weight at station 2 relative to stations 1 and 3. Sample sizes are given in the lower right corner of the panels.

level of either of the two enzymes controlling conversion of testosterone to 11-ketotestosterone.

In males, similar testosterone levels at all sites coincided with similar testicular development in November 1990; there was a similar relationship among sites between gonad weight and carcass weight during this sampling period. In the following spawning season of May 1991, however, male white sucker did exhibit differential relationships in the gain of gonad weight relative to the gain in carcass weight, with reference site 1 differing from exposed site 2; exposed site 3 was similar to sites 1 and 2 (Fig. 6). The lack of consistent differences in the observed responses at stations 2 and 3 relative to the reference station contrasts with similar levels of contamination, MFO induction, and 11-ketotestosterone plasma levels observed among male white sucker from the exposed stations. This observation, in combination with the low $r^2$ values, suggests that variations in gonad weight as a function of carcass weight may be more the result of variations in local environmental conditions than of exposure to effluent.

In females, 17α,20β-diOHprog secretion does not seem to be under a negative influence of xenobiotic effects because circulating levels of this steroid are highest at contaminated sites. However, testosterone, and 17β-estradiol levels were lower in BKME-exposed populations relative to the reference. In females, testosterone and 17β-estradiol are linked, testosterone being the precursor of 17β-estradiol (Scott et al. 1984). Although we know that 17α,20β-diOHprog is involved in the final stage of oocyte maturation immediately prior to spawning (Goetz 1983), we do not know if this hormone plays an active role during early gonadal development. In November, at the reference site, females exhibited a profile of steroid hormones suggesting successful conversion of testosterone to 17β-estradiol.

The limiting factor responsible for a lower level of 17β-estradiol in exposed fish seems to be at least partially due to low testosterone levels, but may also be related to impairment in the biosynthesis of estradiol from testosterone. Low levels of testosterone at exposed sites indicate that a breakdown in the steroid biosynthetic pathway may be occurring somewhere before the synthesis of testosterone in female white sucker. Van Der Kraak et al. (1992) showed that in vitro secretion of testosterone was impaired in eggs originating from BKME-exposed female white sucker. An alternative mechanism explaining differential hormone levels would be increased metabolism of testosterone and 17β-estradiol in exposed females. However, this survey was not designed to differentiate between synthesis and metabolism, but to measure circulating plasma levels. The observed hormonal imbalances may be related to the P-450 system. At present, it is not known if MFO liver enzymes as described by EROD and reproductive steroid hormones are linked in fish. A plausible hypothesis is that both MFO induction and reduced steroid levels are the result of binding by the contaminants to the aryl hydrocarbon (Ah) or to similar receptors, but are not otherwise related (Hodson et al. 1991).

Munkittrick et al. (1991) also observed lowered 17β-estradiol levels in female white sucker from BKME-exposed populations from Lake Superior. However, in contrast with our study, they observed lower levels of testosterone in exposed male white sucker. Hodson et al. (1992) also observed a tendency to decreased testosterone levels in male white sucker from the St. Maurice River in 1989 and slight but nonsignificant increased levels of testosterone and 17β-estradiol levels in BKME-exposed females. However, in that study, fish were sampled in August when steroids were at very low levels. It is unknown whether these different interstudy results reflect seasonal variations in plasma steroid levels or different degrees of impairment.

White sucker undergo gonad maturation in November, but the apparently reduced conversion of steroid precursors to testosterone and 17β-estradiol in females from the St. Maurice River was not reflected in their gonad development during November sampling. Despite lower levels of steroids, females from downstream stations developed gonads to the same extent as reference fish. However, Fig. 6 and 7 indicate that smaller sizes of both males and females are missing from the sample of mature fish, which may reflect an effect of BKME on maturation. Lower levels of testosterone and 17β-estradiol observed in white sucker of the St. Maurice River in November were not necessarily indicative of reproductive impairment during early gonad development of mature fish.

During May 1991, extensive sampling allowed differentiation of gonad development relative to sites. Although all female white sucker exhibited a similar gain in gonad weight relative to a gain in carcass weight in May 1991 (i.e., all curves are parallel), females at reference site 1 had a higher gonad weight than females at exposed site 3 (Fig. 7). We do not know, however, if this small difference in gonad development indicated a biological impact on white sucker populations because fecundity was similar at sites 1 and 3. In addition, fish from site 2 located immediately downstream of the effluent do not appear to be different in their

gonad weight to carcass weight relationships from fish originating from site 1 or 3. Moreover, all fish appeared to have similar mean egg weights. The difference in female gonad development may reflect local environmental conditions prevailing during the reproductive cycle because white sucker are a very phenotypically plastic species (Scott and Crossman 1973).

Although ovarian development was similar at all sites in November of 1990, fecundity differences were observed among the three stations in the following spring. Immediately downstream of the mill, numbers of eggs were poorly correlated with the carcass weight (Fig. 8), while fecundity–weight relationships of fish from reference station 1 and exposed station 3 were statistically similar. The greater variability in the number of developed eggs at site 2 seems to be unrelated to hormone levels, which were altered in a similar fashion at stations 2 and 3. Since only a limited number of reproductive characteristics were measured, it is possible that other mechanisms may be operating.

Despite chemical exposure, enzyme induction, and some reproductive hormonal responses, reproductive impairment was not clearly evident at the level of gonad development, with the exception of increased variability of fecundity–weight relationships immediately downstream of the mill. However, absence of reproductive impairment does not correlate with hormonal variation. In contrast with these results, McMaster et al. (1991) observed that female white sucker exposed to BKME in Jackfish Bay on Lake Superior contained fewer eggs at maturity and exhibited lower gonadosomatic indices, elevated MFO activity, and reductions in plasma steroid levels. In the same area, Munkittrick et al. (1991) also observed delayed sexual maturity and reduced gonad size associated with depressed steroid levels in BKME-exposed white sucker populations. In the same study, however, BKME-exposed female longnose sucker (*Catostomus catostomus*) showed lower steroid levels but similar gonad sizes relative to a reference population. Male longnose sucker from the same contaminated sites did not show any changes in steroid levels or in gonad size compared with a nonexposed population. Munkittrick's and McMaster's study sites were located in a relatively shallow bay of Lake Superior, into which the effluent-carrying creek flows and possibly disperses, creating strong gradients of BKME concentration. The St. Maurice River, however, is a dynamic system in which BKME is rapidly diluted in the river, after which concentrations appear to change little between sites downstream. Therefore, differences in responses of fish between the two study areas may be due to differences in exposure intensity.

The different results from these investigations suggest that elevated MFO activity and altered plasma steroid levels in response to BKME exposure cannot always be associated with obvious reproductive impairment through reduction in gonad size or fecundity. The biochemical and physiological parameters measured in this upstream–downstream study (testosterone, 11-ketotestosterone, 17β-estradiol, 17α,20β-diOHprog, gonad weight, egg weight, and fecundity) do not allow us to clearly relate perturbations in plasma steroid levels to impaired reproduction as measured by gonad weight and fecundity in the St. Maurice BKME-exposed populations of white sucker. However, additional studies are underway to investigate in more detail the effects of pulp and paper mill effluents and natural environmental variations on fish population demographics. Environmental factors may camouflage or modulate fish responses to pulp mill effluents.

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