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RESEARCH AND TECHNOLOGY BRANCH

IMPACT OF BLEACHED KRAFT MILL EFFLUENT (BKME) ON FISH POPULATIONS NEAR TERRACE BAY, ONTARIO

R. A. C. PROJECT NO. 463G and 494G

Prepared for Environment Ontario by:

M. E. McMaster

Environment Ontario

1979

ISBN 0-7729-8828-5

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AUGUST 1991

Cette publication technique n'est disponible qu'en anglais.

PIBS 1656
log 90-9300-463

Acknowledgement and Disclaimer

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The report that follows consists of an executive summary along with five manuscripts that have been submitted for peer review. The project was funded by the Research Advisory Committee of the Ontario Ministry of the Environment as project numbers 463G and 494G. The project proposals were entitled "Impact of bleached kraft mill effluent (BKME) on white sucker populations near Terrace Bay, Ontario" and "Impact of bleached kraft mill effluent on reproductive, biochemical and immunological characteristics of white sucker and whitefish".

This report summarizes results from studies funded through two separate one year RAC grants (463G and 494G) to Dr. D.G. Dixon, at the University of Waterloo in 1989 and 1990. The project was undertaken as a field study designed to determine the impacts of bleached kraft mill effluent (BKME) on fish populations in Jackfish Bay, Lake Superior. Jackfish Bay has been identified as an area of concern (AOC) by the International Joint Commission (IJC) as it receives approximately 121,000 $m^3 d^{-1}$ of BKME from a mill located in Terrace Bay, Ontario. Jackfish Bay has received untreated or primary treated effluent for over 48 years, and only recently (October 1989) has installed a secondary treatment aeration lagoon system. Our data collected in 1989 extends a database initiated in 1988 on the impacts of primary treated effluent on fish populations. Our 1990 collections follow the fish populations for evidence of improvements subsequent to the installation of secondary treatment. White sucker (Catastomus commersoni) are abundant in the region and the majority of the work has been completed on this species. Work was also completed on lake whitefish (Coregonus clupeaformis), and longnose sucker (Catastomus catastomus) populations. All fish data are compared to one of two reference sites, either Mountain Bay or Black Bay, both which have been used previously as reference sites in other studies. The results of these studies have been compiled into a number of different manuscripts that have been submitted for peer review.

During all collections in 1989, male and female white sucker exhibited a decreased length and weight and a higher condition factor at the BKME site. This increased condition factor was consistent with an increased water temperature at the BKME site, but was inconsistent with impacts on growth and reproduction. Both sexes exhibited a decreased growth rate and a shift in size distribution towards smaller fish. Both male and female fish from the BKME site also showed a significantly increased age to maturation and reduced reproductive growth. Using an unbiased sampling technique, both males and females were significantly older at the BKME site.

Reproductive potential was assessed in prespawning (PRES) female white sucker in terms of gonad weight, total fecundity, egg diameter and egg weight. Although there was no difference between sites in mean fecundity, fish from the BKME site were older than those from the reference site. Analysis showed that in fish of the same age, Jackfish Bay females had lower

fecundity especially in young fish. The size of individual eggs in Jackfish Bay females also differed from those in Mountain Bay females. Jackfish Bay eggs were significantly lighter and smaller in diameter than those from the reference site. Eggs in Jackfish Bay females did not increase with age, as was noted at the reference site. The additive result of fewer and smaller eggs at the BKME site, produced a decreased gonadosomatic index (GSI) in Jackfish Bay females.

In spawning (SPAW) fish, males were subjected to a thorough evaluation of reproductive potential. Spermatozoa from Jackfish Bay males showed significantly lower motility, but there was no difference in the spermatocrit values between sites or in the volume of milt released. The seminal plasma constituents measured (potassium, sodium, chloride, and osmolality) were not significantly different between sites. Male fish from the BKME site had significantly reduced secondary sexual characteristics (tubercles) compared to those at the reference site.

Fertilization success was not impaired in white sucker from the BKME contaminated site, as eggs returned to the laboratory showed no difference between crosses within sites or between sites. Eggs were carefully followed until hatch and no difference between hatching time or hatching success was found. Larvae were followed for a period of 70 days post hatch. There was no difference in deformity rate, larval survival, development, behaviour, yolk absorption efficiency, or initiation of exogenous feeding. However, BKME larvae grow at a slower rate when compared to reference larvae.

Reproductive development was assessed in white sucker collected in July and August of 1989. Examination of gonadal condition in July showed no increase in spawning failure at the BKME site. Gonads appeared to be larger and further developed at the reference site in July females, and both males and females in August. Significantly greater GSI's at these sites confirmed the visual appearance.

Testosterone levels were significantly reduced in both prespawning and spawning females at the BKME site. Testosterone, 11-ketotestosterone and 17α,20ß-dihydroxyprogesterone levels were

significantly reduced in exposed males during the prespawning period. July samples showed no differences in female steroid levels, but males showed reduced levels of testosterone. August samples showed reduced testosterone in both females and males relative to two reference sites, as well as reduced 11-ketotestosterone levels in males and reduced 17ß-estradiol levels in females.

Spring fish, both PRES and SPAW, males and females had reduced liver weights at the BKME site. In July and August, the opposite trend was evident as Jackfish Bay fish had significantly increased liver weights compared to the reference sites. The increased liver weights in the summer samples correspond quite well with the increased liver mixed-function oxygenase activity (MFO).

MFO analysis was determined as the activity of aryl hydrocarbon hydroxylase (AHH) activity towards benzo(a)pyrene (B(a)P) and diphenyloxazole (PPO). MFO activity levels measured as a function of PPO activity were higher in all PRES and SPAW males and females at the BKME site. Analysis indicated that only PRES males and SPAW females were significantly different from the controls. Using B(a)P as the substrate, all Jackfish Bay spring fish had increased MFO activity, however analysis showed only PRES males and females to be significantly higher at the BKME site. In July fish, Jackfish Bay males and females had significantly induced MFO activity using both PPO and B(a)P as substrates. August collected white sucker showed a 6- to 18-fold increase in activity towards PPO, and 5-fold increase in B(a)P activity at Jackfish Bay. There was no significant difference in UDPGT activity (glucuronosyl transferase activity towards p-nitrophenol) in the summer samples.

In attempt to link this increased MFO activity to the reduced circulating steroid levels found, steroid injections were attempted in August to monitor the clearance rates of the two populations.. These attempts were unsuccessful due to our inability in keeping the control fish alive. Fish at the BKME site could be captured in a two hour set as the colour of the effluent simulated evening conditions. However, at the control sites overnight sets were required and the stress of being in a net for that length of time was too much for the fish to survive the subsequent

injection and caging experiment. We attempted this study first in the Mountain Bay reference site, then again in Black Bay, however neither attempt was successful.

When BKME exposed fish were removed to clean water for a period of four days, there was a significant reduction in MFO activity (measured with both PPO and B(a)P). Dioxin analysis was completed on our white sucker muscle tissue (Keith Sherman, OME) from the Jackfish Bay site. This data indicates elevated levels of both TCDD and TCDF (tetrachlorodibenzofurans) relative to Mountain Bay fish. However, these levels are substantially lower than those found at other bleached kraft mill sites.

Preliminary work on lake whitefish that were collected from Jackfish Bay, also indicated decreased gonadal growth and increased liver size relative to those at two reference sites. A large number of the BKME fish showed no evidence of gonadal maturation two months prior to the spawning season, indicating an increased age to maturation in whitefish that may exceed that found in the white sucker population.

All data collected in 1989 are presented in the manuscripts

"Changes in hepatic mixed-function oxygenase (MFO) activity, plasma steroid levels and age at maturity of a white sucker (Catostomus commersoni) population exposed to bleached kraft pulp mill effluent" submitted to Aquatic Toxicology (February, 1991),

"Milt characteristics, reproductive performance and larval survival and development of white sucker exposed to bleached kraft mill effluent" submitted to Ecotoxicology and Environmental Safety (June, 1991), and

"External lesions and changes in maturity, MFO activity and plasma sex steroid levels of lake whitefish exposed to bleached kraft mill effluent (BKME)" submitted to Canadian Journal of Fisheries and Aquatic Sciences (June, 1991), or in the MSc. thesis

"Impact of bleached kraft pulp mill effluent on fish populations in Jackfish Bay, Lake

Superior" by Mark McMaster (1991).

White sucker collected from Jackfish Bay during August 1990 exhibited similar hepatic MFO activity as recorded in samples collected during August of 1988 and 1989. Secondary treatment has not been successful in eliminating BKME impacts on MFO activity. Hepatic MFO activity was also induced in both longnose sucker and lake whitefish in August 1990. However, samples collected two weeks after a planned mill maintenance shutdown during September 1990, showed no MFO induction in longnose sucker, reduced MFO activity in white sucker and a reduced impact zone for MFO induction in lake whitefish. Liver size however, did show improvements following the installation of secondary treatment as white sucker livers declined 47% in females and 22% in males and in whitefish by 37% in females and >50% in males (this trend has not been seen in 1991).

A reduction in circulating levels of gonadal sex steroids has been recorded in fish exposed to BKME in Jackfish Bay during 1989. Neither secondary treatment nor mill shutdown were successful in eliminating impacts of BKME exposure on levels of testosterone and 17β-estradiol in female white sucker and longnose sucker. No change in the reduced gonad growth at the BKME site was evident for white sucker and lake whitefish following secondary treatment.

The short duration of MFO induction after shutdown and the persistence of steroid reductions suggest that a) secondary treatment has not been successful in removing "MFO-active" compounds from BKME, b) induction is not related to sediment contamination with persistent compounds, c) the inducing agent(s) are rapidly cleared by fish and that d) effects on steroids may not be directly related to MFO induction. In vivo and in vitro studies in the spring of 1990, suggest that there are other disruptions in the hypothalamic-pituitary-gonadal axis that can account for these lower steroid levels in BKME fish. Although BKME-exposed white sucker are capable of spawning viable eggs, sGnRH failed to induce ovulation in preovulatory fish during a 24 h period, while 10 of 10 fish from the reference site ovulated within 6 h. BKME-exposed fish showed lower plasma levels of both T and 17,20β-P at time 0, while no increase in 17,20β-P was seen after injection of the sGnRH. *In vitro* incubations of ovarian follicles

revealed depressed basal secretion of T and 17,20β-P and diminished responsiveness to human chorionic gonadotropin (hCG). BKME-exposed fish showed lower levels of both free and glucuronated T and 17,20β-P in circulation. These fish however, show similar production of the prostaglandin (PGE_2) in ovarian follicles suggesting that there is no general impairment of ovarian maturation.

The ratio of steroid production between sites is the same as the ratio in blood between sites, suggesting that induced hepatic MFO activity is not associated with altered plasma steroid clearance rates. Independence of hepatic MFO activity and steroidal abnormalities is also suggested by experiments showing a) no change in clearance of injected steroid and b) persistent depression of circulating steroids during spawning and mill shutdown, when MFO levels are not induced.

During our thorough investigation of the lake whitefish populations response to BKME exposure, more than 20% of the whitefish collected at the BKME site exhibited lateral, slash-like lesions which penetrated the body cavity. Histological examination revealed no evidence of an infectious etiology, and the wounds could not be accounted for by known causes. Similar lesions were found in 1991 near a second BKME discharge. The restriction of these lesions to two sites receiving BKME, and the correlation with other adverse impacts suggests that the BKME discharge may be the causative agent.

All results from our 1990 studies and comparisons between the 1989 and 1990 studies are presented in the manuscripts

"Longterm studies of bleached kraft mill effluent (BKME) impact on fish: response of hepatic mixed function oxygenase (MFO) activity and plasma sex steroids to secondary treatment and mill shutdown" submitted to Environmental Toxicology and Chemistry (February, 1991).

"Reproductive dysfunction and MFO activity in three species of fish exposed to bleached kraft mill effluent" submitted to Water Pollution Research Journal of Canada (April, 1991),

"External lesions and changes in maturity, MFO activity and plasma sex steroid levels of lake whitefish exposed to bleached kraft mill effluent (BKME) submitted to Canadian Journal of Fisheries and Aquatic Sciences (June, 1991), and

"Bleached kraft pulp mill effluent (BKME) alters steroid production, regulation and metabolism in white sucker (Catastomus commersoni)" presented in a poster at the Reproductive Physiology of Fish conference (July, 1991).

Changes in hepatic mixed-function oxygenase (MFO) activity, plasma steroid levels and age at maturity of a white sucker (Catostomus commersoni) population exposed to bleached kraft pulp mill effluent.

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Abstract

The impacts of bleached kraft mill effluent (BKME) on a white sucker (Catostomus commersoni) population were examined during May, July and August 1989, and compared with two reference sites. At the time of this study, the effluent received only primary treatment. BKME-exposed white sucker exhibited increased liversomatic indices and elevated mixed-function oxygenase (MFO) activity in both July and August. They also showed lower gonadosomatic indices and an increased age to maturity. The females contained fewer eggs at maturity, while the males had reduced development of secondary sexual characteristics. These fish also had severe reductions in plasma steroid levels throughout the year, including testosterone, and 17α,20β-dihydroxyprogesterone in both sexes, as well as 11-ketotestosterone in males and 17β-estradiol in females. BKME-exposed white sucker were shorter, older and had decreased growth rates compared to those at the reference sites. These fish also exhibited an increased condition factor, yet showed decreased visceral lipid stores. Relative to those at the reference sites, the stomach contents of the BKME-exposed fish revealed reduced numbers of organisms per gut, reduced taxa per gut and increased number of empty stomachs. The decreased energetic commitment to reproduction, along with the increased condition factor, suggests a disruption in metabolic capability and altered energy allocation in fish exposed to BKME.

Keywords: BKME, white sucker, reproduction, MFO activity, steroid hormones, age to maturity

Introduction

There is a growing public awareness and concern about the environmental effects of bleached kraft pulp mill effluent (BKME) (Bonsor et al., 1988). Recent studies have associated BKME with changes in the growth, carbohydrate metabolism, and gonadal maturation of fish, as well as with altered fish community structure (Andersson et al., 1987; Neuman and Karas, 1988; Sandstrom et al., 1988; Bohling et al., 1991; Karas et al., 1991). Mixed-function oxygenase (MFO) induction has been a consistent indicator of BKME exposure in Scandinavia, (Andersson et al., 1987, 1988; Lindstrom-Seppa and Oikari, 1990a,b; Oikari et al., 1985), and has been reported near a number of BKME discharges in Canada (Rogers et al., 1989; Hodson et al., 1991; Munkittrick et al., 1991a,b; Servos et al., 1991; Smith et al., 1991). A preliminary study conducted during 1988 near a bleached kraft mill discharging into Jackfish Bay, Lake Superior, found increased MFO activity and altered steroid profiles in white sucker (Catostomus commersoni) collected during August (Munkittrick et al., 1991a).

MFOs facilitate the excretion of many xenobiotics by enhancing their hydrophilicity through the addition of polar groups. In addition to this role in the elimination of lipophilic contaminants, MFOs are important in the synthesis and metabolism of endogenous compounds including steroids, fatty acids and prostaglandins (Rattner et al., 1989). It has been hypothesized that increased MFO activity may be responsible for the impacts of many lipophilic contaminants on reproduction (Okey, 1990), and impacts on gonadal size and age to maturity have been described at Jackfish Bay (Munkittrick et al., 1991a). This study expanded the preliminary studies in Jackfish Bay to include more detailed sampling, an additional reference site, additional steroid and MFO indicators, and a study of their seasonal variability. The bleached kraft mill discharging into Jackfish Bay installed a secondary effluent treatment system which became operational in September, 1989, just after the completion of this study. As such, this study also presents detailed baseline studies on which to evaluate the performance of secondary treatment in mitigating the impacts of BKME on fish populations.

Materials and Methods

Study Sites

A bleached kraft mill, located in Terrace Bay, Ontario, produces 1200 air-dried metric tonnes of pulp per day and discharges approximately 120,000 m^3 d^{-1} of effluent into the headwaters of Blackbird Creek, which carries the effluent approximately 15 km to Moberley Bay (48° 50'N, 86° 58'W), the western arm of Jackfish

Bay, Lake Superior (Figure 1). Moberley Bay receives no other industrial or municipal effluents and has no permanent residential development.

The mill began operation in 1948 and untreated effluent was discharged until 1978 when two primary treatment clarifiers were installed. The work reported here was completed prior to the installation of a secondary treatment aeration lagoon, which began operation in September, 1989. At the time of this study an effluent plume, characterized by dark colour and high turbidity, was evident for several km from the outfall. The effluent was also markedly warmer than the surrounding water of Lake Superior. Although chemical characterization of the effluent was not available, caging bioassays with rainbow trout (Oncorhynchus mykiss) conducted in 1983 found 100% mortality 1.5 km off the mouth of Blackbird Creek (Flood et al., 1986). Sediment core profiles indicated elevated tetrachlorodibenzofurans (TCDFs) (>2 ng g^{-1}) dating back to 1973 (Sherman et al., 1990).

The reference site(s) for these studies were Mountain Bay (48° 56'N, 87° 50'W) for the spring and July sampling trips as well as Black Bay (48° 30'N, 88° 40'W) for the August trip (Figure 1). These reference sites have been used previously to monitor impacts of BKME (Smith et al., 1991), and neither of these Lake Superior bays receive industrial effluent or significant domestic effluent input.

Sampling Procedures

Prespawning white sucker were collected from spawning streams using overnight hoop net sets in mid-May 1989. At Jackfish Bay, white sucker spawn in tributaries to Jackfish Lake (Figure 1), the only suitable spawning streams within the Jackfish Bay drainage basin. Fish were collected from Sawmill Creek which is not subject to BKME loading. The duration of residency in clean Jackfish Lake water prior to spawning is unknown. At Mountain Bay spawning fish ascend a number of streams; collections were made from the Little Gravel River. Female fish were separated from males and kept in aerated holding tanks filled with stream water until sampling. Male fish were placed in holding nets and left in the streams until immediately prior to sampling. Female fish were sampled between 1030 and 1400 h and male fish from 1500 to 1830 h to minimize variation in blood parameters. Fish collected early in the run, which were still firm, were classified as prespawning, while fish collected further into the spawning season, with ripe, running gametes were sampled as spawning fish.

During July, white sucker were collected with overnight gill net sets (10.0 and 11.3 cm stretch mesh) in the Moberley Bay effluent plume, approximately 500 m from the mouth of Blackbird Creek; August collections

were made with only the 10 cm mesh net. Jackfish Bay is relatively shallow; all fish were collected in approximately 3 to 12 m of water. The surrounding area of Lake Superior drops to more than 60 m in depth, and since white sucker are not typically captured in depths exceeding 12 m in Lake Superior, the white sucker captured in this area during the summer months were considered to be resident. Fish in Mountain Bay and Black Bay were captured in approximately 5-10 m of water. In all cases, live fish were removed from the nets and transported to shore for processing in a 750 L tank of aerated Lake Superior water. The effects of capture stress on the physiology of the fish was assumed to be equal at all sites. All fish were alive when sampled.

Each fish was rendered unconscious by a sharp blow to the head. Fork length (mm) and total weight (g) were determined, and each fish was examined for external lesions. Blood was collected via caudal severance into 5.0 mL heparinized vacuum tubes, stored on ice for 6 to 8 h, and centrifuged prior to the collection of plasma. The liver was excised, weighed (± 0.1 g) and sampled for measurement of MFO activity. Both the plasma and liver samples were immediately frozen in liquid nitrogen and returned to the laboratory where they were stored at -80° C pending analysis. Fish were aged by counting annuli on dried, cleaned opercula from each fish. The gonads were removed and weighed (± 0.1 g), and the ovaries preserved in 10% buffered formalin. In the laboratory, the preserved ovaries were washed, blotted dry and reweighed (± 0.001 g). The number of eggs in triplicate, 1 g samples was determined and these results were used to estimate the total number of eggs per fish (total fecundity). For each fish, the diameter of 10 eggs and the weight of the sample of 10 eggs was also determined in triplicate.

Spawning male fish were rated with respect to the number and distribution of nuptial tubercle expression. The scale ranged from 0 to 6; (0) no tubercles present; (1) 3 to 5 very small tubercles on the anal and/or caudal fin; (2) 3 to 5 very small tubercles on the anal and/or caudal fin and the head; (3) 3 to 5 very small tubercles on the caudal, pelvic and pectoral fins, with tubercles on the head at the eyes and snout only; (4) 5 to 7 tubercles on each ray of the pelvic and pectoral fins, with tubercles on the eyes and snout; (5) not obvious on all fins, but present with the head fully covered; (6) tubercles on all external surfaces. Fish were rated with respect to their visceral lipid stores using a subjective scale ranging from 1 to 5 adapted from Munkittrick and Dixon (1988), (with 1 representing very little visceral lipid and 5 representing large amounts).

Stomach samples were taken from white sucker at all three sites during the August sampling trip. Once the internal organs were removed, the intestine was cut off just below the stomach. Stomach contents were then removed and placed into polyethylene bags filled with 10% formalin. Samples were returned to the laboratory where the contents were identified to the lowest possible taxonomic level. The number of taxa per stomach as well as the number of organisms per stomach (gut abundance) were determined for each

fish.

MFO determinations

MFO activity was determined by catalytic assays using 2,5-diphenyloxazole (PPO), benzo(a)pyrene (B(a)P), and p-nitrophenol (UDPGT) as substrates. PPO determinations were completed after Luxon et al. (1987), B(a)P by the methods of Nebert and Gelboin (1968) and UDPGT activity (in microsomes) after Castren and Oikari (1983). Protein was determined by the Bradford binding assay (Bradford, 1976) with bovine serum albumin as the standard. The activity of the PPO oxygenase is presented as Fluorescence Units min^{-1} mg $protein^{-1}$, which can be related to the fluorescence of a quinine sulphate standard (1 FU equals 0.41 μg quinine sulphate L^{-1} 0.5 N Na OH). The B(a)P oxygenase activity was expressed as nmol of 3-hydroxy-B(a)P min^{-1} mg $protein^{-1}$. UDPGT was determined with 5 mg protein in a reaction mixture of 1 mL, the activity being expressed as nmol p-nitrophenol conjugated min^{-1} mg $protein^{-1}$.

Plasma steroids

Testosterone (T), 17α,20β-dihydroxyprogesterone (DHP), 17β-estradiol (E_2) and 11-ketotestosterone (11KT) were measured by radioimmunoassay (RIA) in plasma following ether extraction. A description of the antisera used to measure T, DHP, E2 and 11KT are reported, respectively, in Van Der Kraak et al. (1984), Wade and Van Der Kraak (1991), Van Der Kraak et al. (1990) and Van Der Kraak et al., (1989). All plasma samples were assayed in duplicate and interassay variability was less than 15% in all four RIA systems.

Statistics

Hoop nets used to capture prespawning (PRES) and spawning (SPAW) fish were considered to be an unbiased sampling of all fish participating in the spawning run. These data are presented separately from the July and August fish which were captured using gill net sets, a biased sampling technique. July and August fish also had to be analyzed separately with respect to size and age as different sizes of gill nets were used in the two collections. There is no reason to expect that length would change with reproductive status (prespawn to spawn), therefore a three-way analysis of variance (ANOVA, site by sex by reproductive status) was used to determine if significant differences existed. Weight, however, changes when gametes are released; comparisons were therefore restricted to two-way ANOVA (site by sex). Site differences in

fecundity were compared using one-way ANOVA of log-transformed values. Non-parametric Mann-Whitney U-tests were used for age, lipid, tubercle, steroid and MFO comparisons between sites. Kruskal Wallis tests were used to determine seasonal differences in MFO activity within sites. The relationships between fork length and age, age and egg weight or egg diameter, as well as the relationships between body weight (total weight minus gonad weight minus liver weight), gonad weight and liver weight, were examined using analysis of covariance (ANCOVA) of log-transformed values, with location of capture as the co-variate. Tukey-Kramer analysis was used to test for differences in the mean abundance of invertebrates in fish guts at the three sites.

Results

There was no difference in the length of fish due to reproductive status (PRES and SPAW) (Table 1), although female fish were longer and heavier than males ($p<0.001$) and reference white sucker were longer than BKME-exposed fish ($p=0.0003$). Condition factor (k) was consistently higher at the BKME site, as indicated by ANCOVA analysis ($p=0.001$). BKME exposed males exhibited lower amounts of visceral fat ($p=0.002$), but females did not differ between sites ($p=0.51$)(Table 1). BKME exposed females were older than reference fish ($p=<0.0001$), while BKME exposed males were older than reference fish only during SPAW ($p=0.003$). During the spawning run, 5 and 6 year old fish made up 57.7% of the males at the reference site, and 39.2% of the females. At the BKME site, these proportions were 26.4% for males and 2.2% for females (Figure 2).

During July, BKME exposed fish were shorter and lighter than those at the reference site (Table 2). In August BKME exposed male white sucker were shorter than Mountain Bay males, but not Black Bay males ($p<0.001$ and $p=0.07$, respectively). There were no differences in length and weight of female fish, or in weight of males between any of the sites in August. Condition factor was consistently higher in white sucker from Jackfish Bay than at either reference site and BKME exposed fish showed lower levels of visceral lipids, except for July males. There were no differences in the ages of fish collected, except for August, when BKME exposed females were older than those from Mountain Bay (Table 2). BKME exposed males and females exhibited a decreased size at age (Figure 3); ANCOVA analysis of age versus length showed no slope differences, significant regressions ($p<0.01$) and intercept differences ($p<0.05$) for all seasons and sexes between sites, except for July males ($p=0.051$).

Compared to reference sites, relative liver size (LSI) was lower at the BKME exposed site in PRES and SPAW fish, but greater in July and August (ANCOVA; Table 3). MFO activity, measured either as a function of PPO

or B(a)P metabolism, was always higher at the BKME exposed site (Table 4). These differences were consistently significant ($p<0.01$) for all summer samples but not for the spring collections. There were seasonal differences in MFO activity in males and females at both sites ($p<0.01$), although changes were not significant in reference males using B(a)P as the substrate ($p=0.084$). Male white sucker had higher MFO activity during PRES and SPAW ($p=0.05$), but during the summer only BKME exposed August samples showed higher activity in males ($p=0.01$). There were no significant differences in uridine-5'-diphosphoglucuronic acid transferase (UDPGT) activity during July or August (Table 4).

Testosterone (T) levels were significantly lower in both sexes at the BKME exposed site during all sampling periods except for SPAW males and July females (Table 5). Female 17α,20β-dihydroxyprogesterone (DHP) levels were significantly lower at the BKME site during SPAW and 17β-estradiol (E_2) levels were lower during August compared to both reference sites. There were no significant differences between sites in female DHP or E_2 levels in PRES female white sucker. Males showed lower levels of 11-ketotestosterone (11KT) during PRES and August collections, and lower levels of DHP in PRES white sucker. DHP levels were higher at the BKME exposed site in both sexes during July (Table 5). Levels of MFO activity (B(a)P) could not be correlated with either liver size ($p>0.60$) or steroid level ($p>0.25$) for males or females at either site.

Although PRES BKME exposed females were older than those in Mountain Bay (Table 1), there was no difference between sites in mean fecundity ($p=0.86$)(Table 3). There were no differences between sites in the slopes of the regressions of age versus fecundity ($p=0.819$), but BKME exposed females had fewer eggs at a given age ($p=0.023$). Eggs at the BKME exposed site were lighter and smaller than those from the reference site ($p<0.001$), with ANCOVA analysis indicating different slopes for both egg diameter and egg weight versus the age of the female, between sites. Both sexes had smaller gonads during all sampling periods at the pulp mill site (Table 3). PRES male fish at the BKME site had fewer nuptial tubercles ($p<0.001$) (Figure 4), however, there were no differences in the levels of tubercle development in female fish between sites ($p=0.73$). Male gonads were not weighed during July due to the small size ($<< 10$ g) and regressed stage of development. Examination of the ovaries in July, showed no difference between sites in the number of females that released their eggs during the spawning season.

Identification and analysis of the white sucker stomach contents from the three sites in August indicated that there were fewer organisms per gut in BKME exposed fish stomachs compared to both reference sites ($p=0.02$), with no difference between the two reference sites ($p=0.502$) (Table 6). The average number of taxa per stomach was also reduced at the BKME exposed site, with an increased number of empty stomachs found. There was also a trend from pollution sensitive species to that of pollution tolerant. The stomach contents showed a shift in community structure whereby pollution sensitive groups such as

Ephemeroptera, Trichoptera, and Amphipoda were replaced by pollution tolerant organisms such as Chironomidae and Isopoda.

Discussion

BKME-exposed white sucker were consistently older and shorter, had an increased condition factor, and had lower visceral lipid stores relative to unexposed fish. Stomach contents of white sucker from the BKME site had a significantly reduced abundance of invertebrates per gut, reduced number of taxa per gut and an increased occurrence of empty stomachs. Changes in benthic species have been previously recorded in Jackfish Bay (Farara et al., 1988). Other studies conducted prior to the initiation of secondary treatment at Jackfish Bay have concluded that "a 5 km^2 area of Moberley Bay and Jackfish Bay has been altered to an extent that normal clean water bottom dwelling organisms are not able to survive and have been replaced by pollution-tolerant forms. This is a result of direct toxic influences of the effluent or deposition of organic wastes" (IJC, 1989). Increased condition factor is normally associated with an increased food availability, and should be associated with an increase in growth rate and fecundity (Munkittrick and Dixon, 1989) at the BKME site. These changes at Jackfish Bay were associated with a decreased food availability and a decrease in growth rate and age-specific fecundity.

The increased condition factor of BKME-exposed fish suggests that the differences in growth and gonadal investment may be related to metabolic redistribution rather than to food limitation. Sandstrom et al. (1988) suggested that excess energy could be manifested as a compensating increase in length, an increased storage, and or growth of reproductive tissue. The BKME fish from Jackfish Bay showed a decreased energetic commitment to growth, visceral lipid storage and reproduction, suggesting a disruption in metabolic capability and altered energy allocation in fish exposed to BKME. The increased condition may be due to increased muscle or liver lipids.

BKME collections had very few spawning females below the age of 8, while reference collections showed a large number of 5, 6 and 7 year old fish. Although fish matured at an older age, Munkittrick et al. (1991a), found that these fish, both males and females, matured at a decreased size. Size at age corresponded closely with data reported for fish collected in 1988 (Munkittrick et al., 1991a), which also indicated that Jackfish Bay white sucker were shorter than white sucker from three areas of Lake Superior. White sucker eggs collected from Jackfish Bay, and reared in clean water, showed no significant differences in growth to the age of 27 days post-hatch (McMaster et al., 1991a). It is unknown how long the white sucker juveniles spend in effluent-free water, or at what age the fish move into Jackfish Bay.

Fish from the BKME site had increased mixed-function oxygenase (MFO) activity during all collections using both PPO and B(a)P as substrates. These fish also exhibit a 17-fold induction of ethoxyresorufin-o-deethylase (EROD) activity (Munkittrick et al., 1991b). While these findings are consistent with other studies showing induction of EROD and B(a)P-hydroxylase (aryl hyrdocarbon hydroxylase; AHH) in fish exposed to BKME (Rogers et al., 1989; Hodson et al., 1991; Munkittrick et al., 1991a; Servos et al., 1991; Smith et al., 1991), the physiological significance of environmental induction is not yet known, but may increase tolerance to pollutants (Stegeman and Kloepper-Sams, 1987).

As with our earlier study (Munkittrick et al., 1991a), MFO induction was reduced or eliminated during the spawning period. It is not clear whether this reduction is due solely to normal seasonal changes or to migration of the fish from the BKME-contaminated site to an uncontaminated site (Sawmill Creek) for spawning. Although natural seasonal changes in MFO activity have been widely reported (i.e. Lindstrom-Seppa, 1985), persistence of MFO induction has been reported in starry flounder (Platichthys stellatus; Spies et al., 1988) and white sucker (I.R. Smith, unpubl. data) spawning in contaminated areas. Sloof et al. (1983) attributed a decline in enzyme activity at spawning to a decrease in contaminant levels due to dilution and removal of the contaminant source.

Male MFO activity was higher than that in females at both sites during all spring sampling periods. This difference was eliminated in the summer collections except in August Jackfish Bay white sucker when males were higher. Forlin and Andersson (1984) concluded that MFO's and total cytochrome P-450 content are generally higher in mature male fish than in mature female fish due to the influence of gonadal steroids. Administration of 17β-estradiol to juvenile rainbow trout prior to treatment with Clophen A50, decreased the degree of MFO induction but testosterone had no effect. Stegeman and Kloepper-Sams (1987) found that this sex difference was larger in fish than in mammals and that estradiol seemed to be the regulator in fish while testosterone was in mammals.

Jackfish Bay white sucker had no difference in levels of uridine-5'-diphosphoglucuronic acid transferase (UDPGT) activity in the summer samples. The response of these phase II enzymes to BKME however, has not been consistent. Smith et al., (1991) found no change in white sucker UDPGT activity near BKME, while Scandinavian studies have found phase II or conjugation enzymes to be reduced by BKME exposure to rainbow trout (Oikari et al., 1985). These increases have been associated predominantly with high levels of resin acids (Tana, 1988). Other Scandinavian studies have found increased UDPGT activity in rainbow trout caged in pulp mill effluent (Lindstrom-Seppa and Oikari, 1990b). UDPGT activity has been found to vary with the site and species tested (Lindstrom-Seppa and Oikari, 1990b) which may explain differences found.

BKME exposed fish had increased liver size during the summer sampling periods, as well as an increase in MFO activity. However, there were no correlations within site between MFO activity and liver size for individual fish. Contaminant levels in BKME fish were elevated in comparison to our reference site. Dioxin analysis of white sucker muscle tissue from Jackfish Bay found levels of total tetrachlorodibenzo-p-dioxin (TCDD) to be 7 pg g^{-1} of TCDD and 44 pg g^{-1} for tetrachlorodibenzofuran (TCDF) (n=4; 1.73% lipid) (Sherman et al., 1990). Increased liver size associated with environmental contamination has been correlated with increased enzyme activity and hypertrophy of liver cells in mature fish (Sloof et al. 1983; Elskus and Stegeman, 1989) and hyperplasia in younger fish (Sloof et al., 1983). Increased liver size associated with a high carbohydrate diet resulted in carbohydrate storage and altered hepatic metabolism of copper (Dixon and Hilton, 1985).

No significant reductions were seen in plasma steroid levels of July BKME exposed females, when gonads were fully regressed, although steroid reductions were evident at the BKME site during all other sampling periods, similar to the preliminary study (Munkittrick et al., 1991a). These changes correlated with increased age to maturity, decreased gonadal size and decreased secondary sexual characteristics in male fish. Eggs failed to increase in size with age at the BKME site, as was noted at the reference site. This was also found in work by Munkittrick and Dixon (1988) in fish exposed to elevated levels of copper and zinc. Although eggs were smaller at the BKME exposed site, this had no effect on egg hatchability, initial larval size or larval survival (McMaster et al., 1991a). Springate and Bromage (1985) found that there was a positive correlation between the size of the egg and that of the fry in rainbow trout. That is the larger the egg (measured after water hardening), the larger the fry at hatch. This relationship was lost however, four weeks after first feeding and no effect of egg size on eye up, hatch, swim up and survival was found. Although male gonads were smaller in August, there was no significant difference at spawning time, and no differences were found between sites for milt volume, spermatocrit levels, or seminal plasma constituents; BKME males did have reduced spermatozoan motility (McMaster et al., 1991a).

In this study, increased MFO activity corresponded with decreased levels of circulating steroid hormones. Since steroids are a natural substrate for certain MFO isozymes (Payne et al., 1987), there is the possibility that high levels of MFO activity may result in a decrease in circulating steroid levels (Okey, 1990) or a shift in their circulating forms, to different metabolites. In vitro incubations of liver microsomes from juvenile rainbow trout have been shown to hydroxylate E_2 into esterone, 6α and 6β-hydroxylated estrogens (Hansson and Rafter, 1983). Studies have found that induction of the MFO system by inducing compounds such as β-naphthoflavone, polychlorinated biphenyls or 3-methylcholanthrene have increased hepatic microsomal metabolism of androstenedione (Hansson and Rafter, 1983) and 17β-[^{3}H]estradiol (Forlin and Haux, 1985). Other studies injecting TCDD and BKME alone or in combination found increased MFO activity in all fish

injected with TCDD, but no differences in circulating testosterone levels (Lindstrom-Seppa and Oikari, 1989). The major inducible AHH isozymes associated with BKME exposure have not been shown to significantly metabolize steroids in vitro (Stegeman and Kloepper-Sams, 1987) and the induction of EROD by β-napthoflavone does not induce estradiol metabolism (Snowberger and Stegeman, 1987). The level of MFO activity could not be correlated with steroid levels for individual fish.

In a number of studies involving PCBs and PAHs authors have found increased MFO activity and reduced steroid hormone levels or reduced reproductive performance in fish (Sivarajah et al., 1978; Spies et al., 1988). Although no direct link can be made between the two, it was thought that these induced MFO systems also increase the metabolism of the endogenous steroid substrates (Okey, 1990). Recent work by our group has shown that the reproductive system in fish exposed to BKME is altered at a number of levels in the hypothalamic-pituitary-gonad axis, including altered response to administration of an exogenous GnRH analogue, altered in vitro steroid production by ovarian tissue and altered peripheral steroid metabolism (G. Van Der Kraak, unpubl. data). Although the phenomenon of MFO induction and steroid depression are often found together, the phenomena may be separate and distinct reactions to unidentified contaminants.

The bleached kraft mill at Terrace Bay mill has installed a secondary treatment aeration lagoon, which began operation immediately after the completion of this study (September, 1989). Preliminary results from 1990 collections have shown reduced BOD and suspended solid loadings to Jackfish Bay, reduced temperature influence and elimination of acute toxicity problems. Work on the fish populations in Jackfish Bay is ongoing to document recovery time of the Jackfish Bay ecosystem.

Acknowledgements

MFO measurements were conducted by Ms. Janine Clemons. Funding for this study was provided by the Research Advisory Council, Ontario Ministry of Environment. Field collections were supervised by Leo and Maye Marchand (Marchand Fisheries), and the assistance of Ms. Linda Melnyk-Ferguson Ontario Ministry of Natural Resources (OMNR), Terrace Bay, Ontario and Mr. Lorne Townes OMNR, Nipigon, Ontario are gratefully acknowledged. This report was prepared for the Ontario Ministry of Environment (MOE), as part of a MOE-funded project related to Jackfish Bay. The views and ideas expressed in this report are those of the authors and do not necessarily reflect the views and policies of the Ontario provincial or Canadian federal government, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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Table 1. Length, weight, condition factor, age and visceral lipid index of prespawning and spawning white sucker at a BKME exposed (Jackfish Bay) and a reference (Mountain Bay) site. Values are given as mean ± s.e (n). Within columns, values with a common alphabetical superscript are not significantly different ($p<0.05$).

Site	Length (cm)	Weight (g)	Condition Factor[1]	Age (y)	Lipid
Prespawning Males					
Jackfish	36.2±0.57 (15)[a]	698±34.4 (15)[a]	1.46±0.02 (15)	8.33±0.67 (15)[a]	
Mountain	39.0±0.63 (15)[b]	780±38.4 (15)[a]	1.30±0.02 (15)*	8.07±0.77 (14)[a]	
Prespawning Females					
Jackfish	40.4±0.49 (25)[c]	993±40.9 (25)[b]	1.49±0.02 (25)	10.32±0.59 (25)[c]	
Mountain	41.1±0.56 (26)[d]	973±39.9 (26)[b]	1.39±0.01 (26)*	7.23±0.46 (26)[d]	
Spawning males					
Jackfish	37.0±0.46 (23)[a]	697±23.8 (23)[a]	1.37±0.02 (23)	8.52±0.45 (23)[a]	2.0 ± 0.1 (23)[a]
Mountain	37.9±0.44 (30)[b]	691±21.3 (31)[a]	1.27±0.02 (30)*	6.84±0.37 (31)[b]	2.7 ± 0.1 (30)[b]
Spawning females					
Jackfish	41.1±0.74 (20)[c]	1009±52.8 (20)[b]	1.44±0.03 (20)	11.25±0.89 (20)[c]	2.3 ± 0.2 (19)[a]
Mountain	43.0±0.94 (20)[d]	1137±78.5 (20)[b]	1.39±0.04 (20)	9.85±1.05 (20)[a]	2.4 ± 0.2 (20)[a]

[1] Difference not compared between seasons or sexes, analysis by ANCOVA of log length vs log body weight by site

* Significantly different intercepts ($p<0.05$)

Table 2. Length, weight, condition factor, age and visceral lipid index of July and August white sucker exposed to BKME (Jackfish Bay) and two reference (Mountain Bay and Black Bay) sites. Values are given as mean ± s.e. (n). Within columns and months, values without a common alphabetical superscript are significantly different ($p<0.05$).

Site	Length (cm) [1]	Weight (g)	Condition Factor[2]	Age (y)	Lipid
July males					
Jackfish	39.7±0.31 (16)[a]	922±24.1 (16)[a]	1.47±0.03 (16)	11.38±0.46 (16)	2.4±0.2 (16)
Mountain	41.9±0.55 (15)[b]	1003±37.5 (15)[b]	1.36±0.02 (15)	13.27±1.63 (15)	2.8±0.3 (15)
July females					
Jackfish	41.0±0.40 (32)[a]	999±29.9 (32)[a]	1.44±0.02 (32)	10.90±0.53 (32)	3.0±0.2 (16)[a]
Mountain	42.9±0.66 (28)[b]	1091±43.3 (28)[b]	1.38±0.02 (28)	10.21±0.89 (28)	4.1±0.2 (15)[b]
August males					
Jackfish	39.4±0.59 (11)[a]	977±47.4 (11)	1.58±0.03 (11)*	12.27±1.17 (11)	2.6±0.3 (11)[a]
Mountain	42.6±0.54 (10)[b]	1064±39.8 (9)	1.40±0.03 (9)	11.60±1.01 (10)	3.3±0.3 (10)[b]
Black	40.8±0.47 (15)[a]	998±30.7 (15)	1.47±0.03 (15)	10.13±0.71 (15)	3.8±0.2 (15)[b]
August females					
Jackfish	40.6±0.50 (15)	996±36.7 (15)	1.48±0.03 (15)	10.67±0.60 (15)[a]	3.6±0.2 (15)[a]
Mountain	41.7±0.89 (14)	1005±64.7 (14)	1.37±0.02 (14)	7.86±0.99 (14)[b]	4.5±0.2 (14)[b]
Black	42.6±0.79 (15)	1088±48.6 (15)	1.40±0.03 (15)	9.64±0.82 (14)[a]	4.4±0.2 (15)[b]

[1] Male and female lengths analyzed separately

[2] Difference not compared between seasons or sexes, analysis by ANCOVA of log length vs log body weight by site

* Significantly different intercepts ($p<0.05$)

Table 3. Liversomatic indices, gonadosomatic indices and fecundity of white sucker exposed to BKME (Jackfish Bay) relative to reference site(s) (Mountain Bay and Black Bay). Within columns, values with a common alphabetical superscript are not significantly different ($p<0.05$)

Season	Sex	Site	LSI	GSI	Fecundity
PRES	Male	Jackfish	2.18 ± 0.07 (15)[a]	4.51 ± 0.26 (15)[a]	
		Mountain	2.46 ± 0.15 (15)[b]	5.52 ± 0.28 (14)[a]	
	Female	Jackfish	2.53 ± 0.09 (14)[a]	12.1 ± 0.59 (24)[*]	24322 ± 1381 (24)
		Mountain	2.88 ± 0.08 (15)[b]	15.5 ± 0.60 (26)	24388 ± 1164 (26)
SPAW	Male	Jackfish	1.75 ± 0.06 (15)[*]		
		Mountain	2.13 ± 0.10 (15)		
	Female	Jackfish	1.68 ± 0.06 (15)[a]		
		Mountain	1.81 ± 0.08 (17)[a]		
July	Male	Jackfish	2.82 ± 0.23 (15)[a]		
		Mountain	1.85 ± 0.11 (15)[b]		
	Female	Jackfish	2.70 ± 0.24 (15)[a]	1.74 ± 0.08 (15)[a]	
		Mountain	2.11 ± 0.12 (15)[b]	2.34 ± 0.11 (15)[b]	
August	Male	Jackfish	2.74 ± 0.24 (11)[*]	3.47 ± 0.53 (11)[1]	
		Mountain	1.39 ± 0.09 (8)	5.54 ± 0.47 (9)	
		Black	1.52 ± 0.08 (15)	6.93 ± 0.33 (15)	
	Female	Jackfish	3.65 ± 0.18 (15)[a]	2.66 ± 0.16 (15)[a]	
		Mountain	1.58 ± 0.10 (14)[b]	2.78 ± 0.14 (14)[a]	
		Black	1.81 ± 0.08 (15)[b]	3.63 ± 0.15 (15)[b]	

Differences not compared between seasons or sexes, analysis by ANCOVA of liver weight or gonad weight versus of body weight by site

[*] ANCOVA indicated different slopes or [1] no significant regression

Table 4. Mixed function oxygenase activity (mean ± s.e. (n)) of PMS supernatant (PPO) or microsomal fraction (B(a)P, UDPGT) from homogenized livers of white sucker captured in the spring (PRES and SPAW), July and in August, from Jackfish Bay or its tributary (BKME site), Mountain Bay or its tributary and Black Bay (reference sites).

Sex	Site	Prespawn	Spawn	July	August
(FU mg^{-1} min^{-1})					
Male	Jackfish	6.89 ± 1.74 (6)	7.95 ± 1.76 (6)	22.17 ± 3.43 (6)	22.23 ± 3.40 (6)
	Mountain	3.20 ± 0.44 (6)**	2.65 ± 0.46 (6)	2.03 ± 0.11 (6)**	1.41 ± 0.16 (6)**
	Black				1.93 ± 0.23 (6)**
Female	Jackfish	1.74 ± 0.54 (6)	2.28 ± 0.41 (6)	32.84 ± 4.57 (6)	11.94 ± 0.78 (6)
	Mountain	0.76 ± 0.13 (6)	1.14 ± 0.21 (8)*	1.61 ± 0.20 (6)**	1.64 ± 0.21 (6)**
	Black				1.84 ± 0.15 (6)**
BaP (nmol (3-OH-BaP) mg^{-1} min^{-1})					
Male	Jackfish	131.8 ± 16.6 (6)	94.1 ± 15.9 (6)	244.3 ± 37.8 (6)	255.1 ± 33.9 (6)
	Mountain	30.1 ± 3.2 (6)**	51.2 ± 9.4 (6)	49.9 ± 5.9 (6)**	57.9 ± 9.8 (6)***
	Black				22.4 ± 5.1 (6)***
Female	Jackfish	47.8 ± 11.9 (6)	39.1 ± 7.5 (6)	280.6 ± 28.2 (6)	168.7 ± 16.8 (6)
	Mountain	15.1 ± 1.7 (6)*	29.1 ± 4.1 (8)	61.6 ± 10.4 (6)**	46.1 ± 10.5 (6)**
	Black				26.5 ± 3.5 (6)**
UDPGT (nmol mg^{-1} min^{-1})					
Male	Jackfish			0.142 ± 0.024 (4)	0.144 ± 0.036 (4)
	Mountain			0.057 ± 0.020 (2)	0.116 ± 0.026 (6)
Female	Jackfish			0.156 ± 0.044 (6)	0.112 ± 0.023 (5)
	Mountain				0.177 ± 0.032 (6)

* $p<0.05$; ** $p<0.01$; *** $p<0.001$

Table 5. Testosterone, 17ß-Estradiol, 11-Ketotestosterone, and 17α,20ß-dihydroxyprogesterone levels in white sucker exposed to BKME (Jackfish Bay) and reference site(s) (Mountain Bay and Black Bay). Values are given as mean ±s.e.(n) in pg mL^{-1} of plasma steroid.

Season	Sex	Site	Testosterone	17-ßEstradiol	11-Ketotestosterone	17α,20ß-Dihydroxyprogesterone
PRES	Males	Jackfish	2798 ± 383 (10)		50019 ± 7991 (10)	611 ± 48.0 (10)
		Mountain	7661 ± 1069 (10)***		107849 ± 8446 (10)**	1233 ± 257 (10)*
	Females	Jackfish	4848 ± 952 (10)	1956 ± 449.0 (10)		463 ± 59.1 (10)
		Mountain	13777 ± 4030 (10)*	1787 ± 328.7 (10)		448 ± 47.0 (10)
SPAW	Males	Jackfish	2938 ± 763 (9)			1703 ± 270 (10)
		Mountain	2775 ± 360 (5)			1254 ± 108 (5)
	Females	Jackfish	762 ± 228 (10)			2500 ± 878 (10)
		Mountain	4098 ± 1815 (6)**			6604 ± 1693 (6)*
July	Males	Jackfish	141 ± 17 (10)		162 ± 50.9 (7)	1513 ± 119 (10)
		Mountain	298 ± 62 (10)*		395 ± 130 (10)	862 ± 132 (10)**
	Females	Jackfish	168 ± 37 (10)	164 ± 28.6 (10)		667 ± 28.1 (10)
		Mountain	154 ± 41 (10)	182 ± 18.7 (10)		542 ± 28.4 (10)**
August	Males	Jackfish	254 ± 66 (10)		463 ± 129 (10)	
		Mountain	639 ± 77 (10)**		1591 ± 135 (10)**	
		Black	342 ± 55 (10)		1138 ± 125 (10)**	
	Females	Jackfish	179 ± 25 (10)	238 ± 53.8 (10)		
		Mountain	398 ± 49 (10)*	893 ± 241 (10)***		
		Black	218 ± 40 (10)	897 ± 152 (10)***		

* $p<0.05$; ** $p<0.01$; *** $p<0.001$

Table 6. Summary data for invertebrates[1] identified from white sucker stomachs taken from Jackfish Bay, Mountain Bay, and Black Bay. Detailed data in McMaster (1991b).

Site	Sample size	Mean number of taxa per gut	Mean abundance per gut	Number of empty guts
Jackfish Bay	18	2.3	17.7	6
Mountain Bay	23	10.3	56.8*	2
Black Bay	25	8.6	81.0***	0

[1] Does not include Pelecypoda, Gastropoda, or Oligochaeta. * $p<0.05$; *** $p\leq 0.001$;

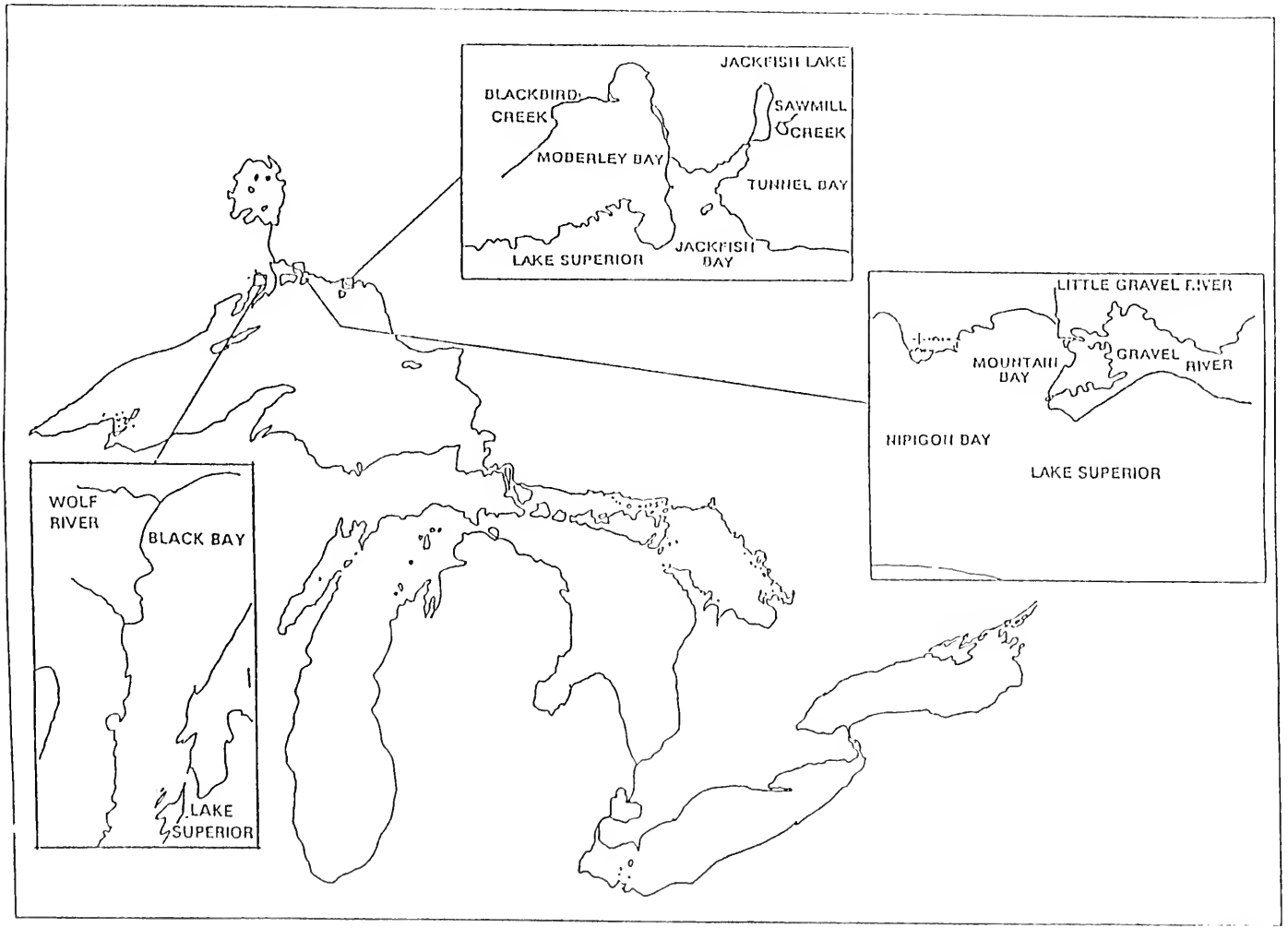
Legend to figures

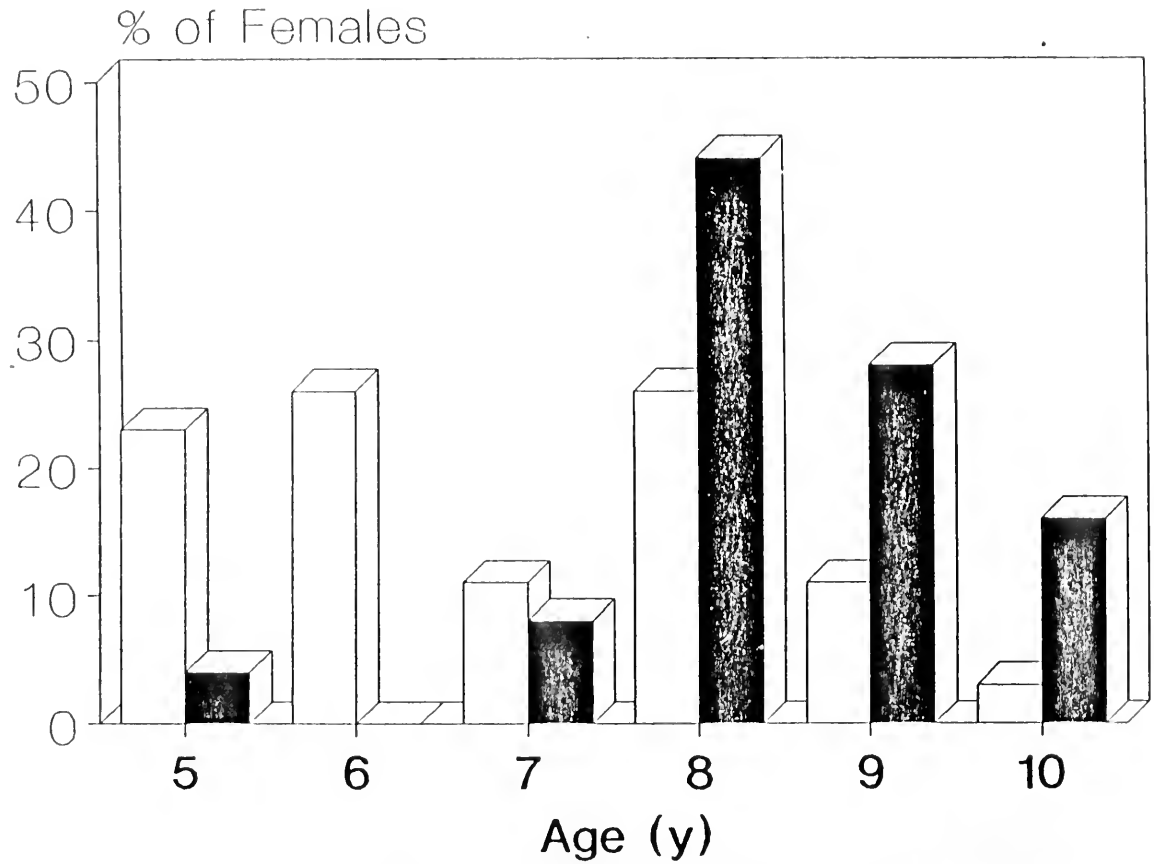
Figure 1 The Study Site. Effluent from the bleached kraft mill at Terrace Bay, Ontario reaches Lake Superior at Jackfish Bay, with Mountain Bay and Black Bay serving as reference sites.

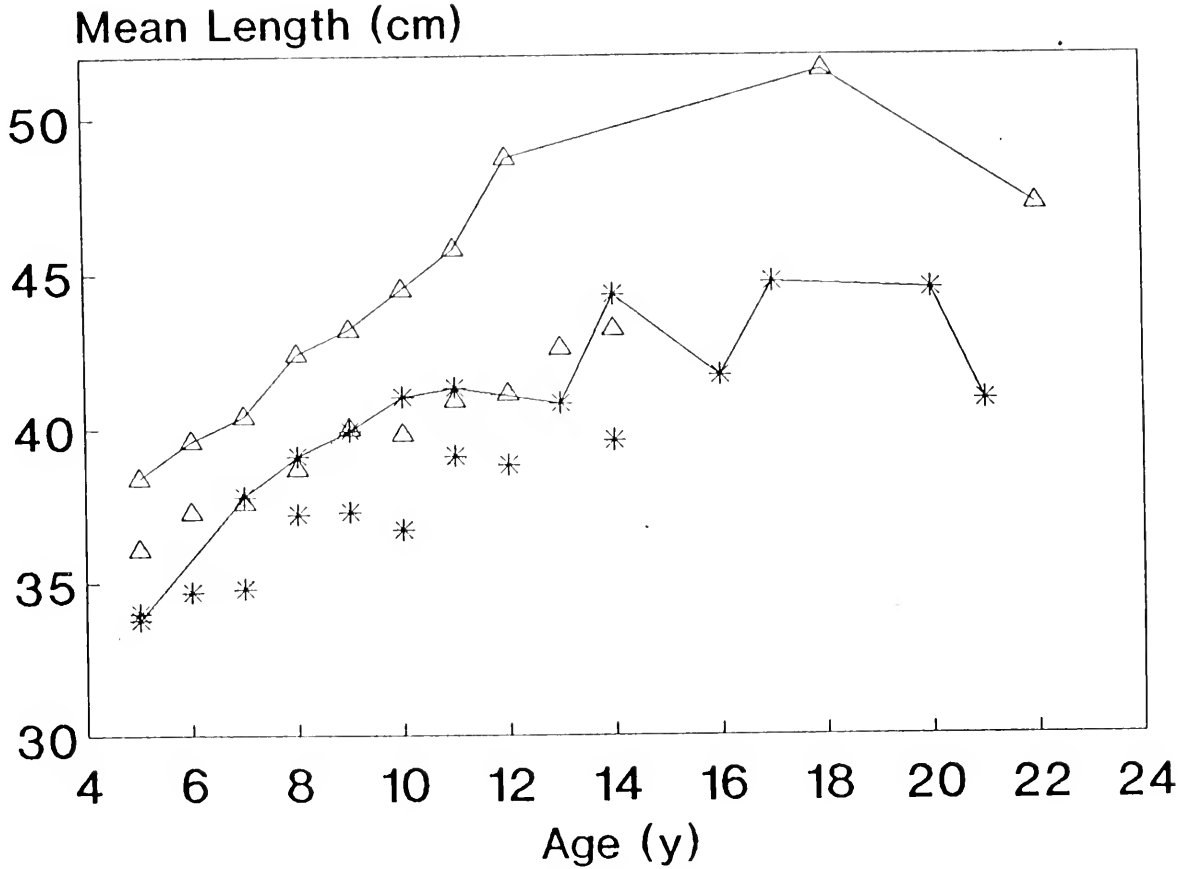
Figure 2 Percentage of female white sucker entering the spawning streams below the age of 10 at the BKME exposed (Jackfish Bay; solid bars) and the reference (Mountain Bay; open bars) sites.

Figure 3 Length at age for spawning male (dotted lines) and female (solid lines) white sucker from the BKME exposed site (Jackfish Bay; asterisks) and the reference population (Mountain Bay; triangles).

Figure 4 Tubercle development in white sucker collected during the spawning period at the BKME exposed (Jackfish Bay; solid bars) and control (Mountain Bay; open bars) locations, indicating reduced secondary sexual characteristics.


JACKFISH LAKE
BLACKBIRD CREEK
SAWMILL CREEK
MODERLEY BAY
TUNNEL BAY
LAKE SUPERIOR
JACKFISH BAY
LITTLE GRAVEL RIVER
MOUNTAIN BAY
GRAVEL RIVER
NIPIGON BAY
LAKE SUPERIOR
WOLF RIVER
BLACK BAY
LAKE SUPERIOR


% of Females
50
40
30
20
10
0
5
6
7
8
9
10
Age (y)


Mean Length (cm)
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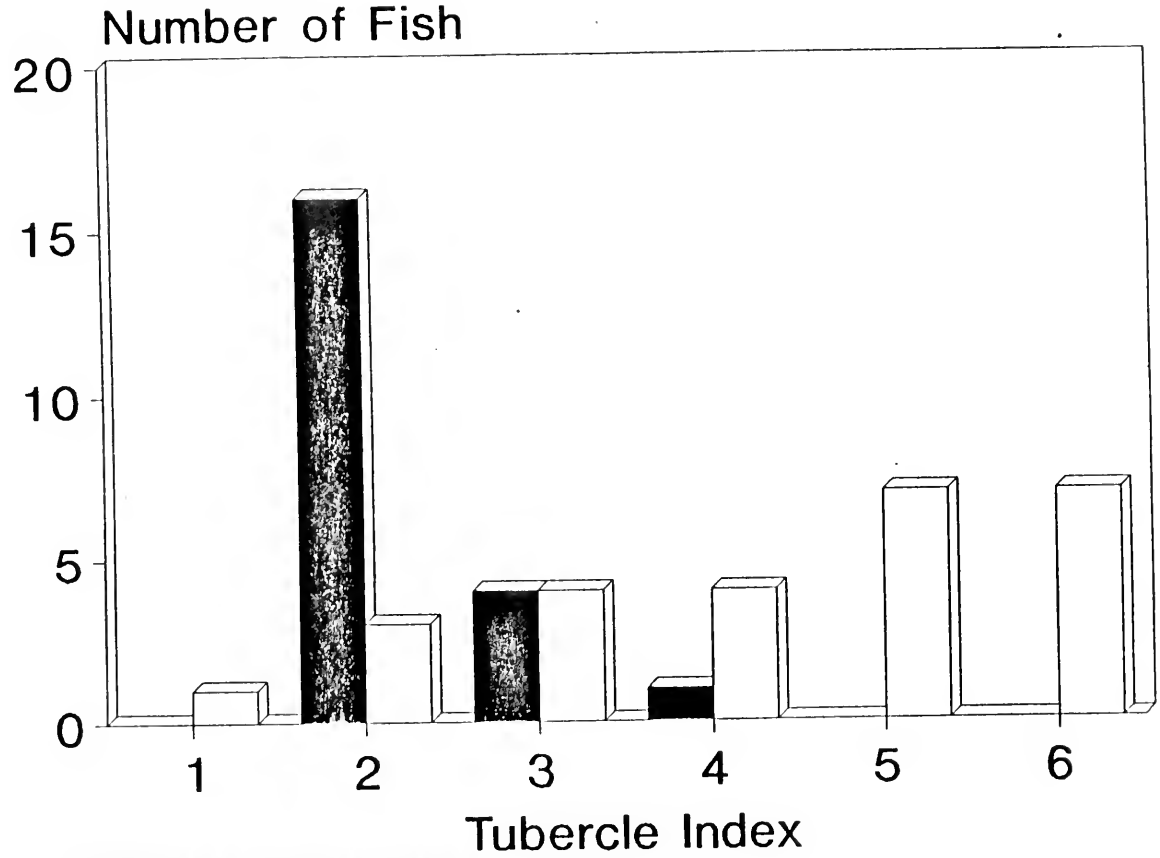
45
40
35
30
4
6
8
10
12
14
16
18
20
22
24
Age (y)


Number of Fish
20
15
10
5
0
1
2
3
4
5
6
Tubercle Index

Milt characteristics, reproductive performance and larval survival and development of white sucker exposed to bleached kraft mill effluent.

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ABSTRACT

White sucker from a Lake Superior bay which receives bleached kraft mill effluent (BKME) show increased hepatic MFO activity, reduced plasma sex steroid levels, decreased egg and gonad size, a decrease in the occurrence of secondary sexual characteristics and an increased age to maturation. This study evaluated the reproductive performance of that white sucker population relative to a similar reference population. Spawning male white sucker from the BKME site had reduced spermatozoan motility but no significant differences in milt volume, spermatocrit levels or seminal plasma constituents. BKME male and female fish had equal or greater fertilization potential compared to both male and female fish at the reference site. There was no difference in either the hatchabilty of the eggs or in larval size at hatch. BKME larvae did show reduced growth rates by 24 days posthatch but showed equal rates of yolk utilization. No difference in larval MFO activity was detected between sites at 21 d posthatch, indicating no parental transfer of induction to the progeny.

INTRODUCTION

There is little knowledge of the impact of untreated or treated whole pulp mill effluent on fish reproduction, and the survival and performance of early developmental life stages (McLeay, 1987). Studies in the Baltic Sea have shown significantly lower numbers of fish larvae and fry near discharges of bleached kraft pulp mill effluent (BKME) (Neuman and Karas, 1988). Other Baltic studies have shown reductions in the overall size of the reproductive areas (reviewed in Sodergren, 1989). Whether the decreased larval numbers in the Baltic Sea are due to increased mortality of sensitive early life stages, or to impacts on gonadal development of the parent fish, is unknown (Sodergren et al., 1988).

Adult fish had markedly reduced reproductive capacities, as gonads failed to develop normally, and they also showed increased hepatic mixed-function oxygenase (MFO) activity (Andersson et al., 1987; Sandstrom et al., 1988). Spies et al. (1985) found a significant negative correlation between MFO activity and fertilization success in starry flounder (*Platichthys stellatus*). Since steroid compounds are natural substrates for MFO enzymes, the synthesis and metabolism of steroids by induced MFO systems could theoretically result in effects on fish reproduction (Truscott et al., 1983; Okey, 1990). Another possible reason for the observed decreases in larval fish may be increased larval mortality. Early life stages are more sensitive to a number of environmental contaminants (Van Leeuwen et al., 1985). Furthermore, it is possible for lipophilic contaminants to be transferred from maternal sources to the eggs (Niimi, 1983), and for these compounds to show bioactivity (Binder and Lech, 1984; Binder and Stegeman 1984).

For the full evaluation of the aquatic toxicity of a chemical, life cycle tests are required. These tests are often carried out with artificially fertilized eggs from fish reared in uncontaminated water which are then exposed to the chemical in question (Van Leeuwen et al., 1985). At Jackfish Bay, Lake Superior, white sucker adults are exposed to BKME throughout the year. These fish have reduced gonad size, increased mixed-function oxygenase (MFO) activity, decreased plasma sex steroid levels, increased age to maturation, and reduced secondary sexual characteristics in male fish. Although these symptoms are

present, there is no difference in mean fecundity of the females, and there has been no evidence of failure to spawn or altered efficiency of egg release. Similar effects on age to maturation, MFO activity, and steroid levels are evident in lake whitefish (*Coregonus clupeaformis*) populations at Jackfish Bay (Munkittrick et al., 1991c).

When spawning season approaches, the white sucker travel out of the exposure area into streams uncontaminated by BKME to release their gametes. These eggs incubate and hatch, and the larvae grow, in these uncontaminated waters. Although the length of time which juvenile white sucker spend in these areas is unknown, the most sensitive stages of their development are spent in clean water. This may be of great benefit to the white sucker populations in this area. Studies have shown that incubation in various concentrations of BKME, increases the mortality of pike (*Esox lucius*) eggs from control areas (Tana and Nikunen, 1986). Whether fish that leave contaminated areas to spawn encounter similar problems is unknown. After uptake, highly lipophilic compounds are stored quickly in lipid deposits, where they may be unavailable for interaction with other tissues except under conditions of lipid mobilization (starvation, ovarian maturation). This allows lipophilic xenobiotics which enter the female from the surrounding water and feed to accumulate in the egg cells if not metabolized (Van Leeuwen et al., 1985). Although the chemical composition of BKME is unknown, white sucker from Jackfish Bay contain elevated levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin and 2,3,7,8-tetrachloro-dibenzofuran (Sherman et al., 1990). These chemicals have been shown to be toxic to fish and may be passed on to the progeny during reproductive growth.

This study was designed to determine if white sucker exposed to BKME show any evidence of altered reproductive performance or larval survival. White sucker were collected and subjected to a thorough evaluation of fertility, gamete quality and larval development and growth. Reproductive potential was assessed in males (milt volume, spermatocrit, motility, seminal plasma constituents) and females (fertility). Fertilized eggs were returned to the laboratory and evaluated for effects on development, growth, behaviour, yolk absorption efficiency, deformation and initiation of exogenous feeding. MFO activity was measured in larvae following the development of the liver.

MATERIALS AND METHODS

Jackfish Bay, located along the north shore of Lake Superior, receives BKME from a mill located in Terrace Bay, Ontario. Production of approximately 1200 air dried metric tonnes of pulp per day results in the discharge of approximately 121,000 $m^3 d^{-1}$ of effluent into the headwaters of Blackbird Creek. The creek receives little dilution between the discharge from the mill, prior to its entry into Moberley Bay (48°50'N, 86°58'W), the western arm of Jackfish Bay (Figure 1), a distance of approximately 15 km. Over the last 40 years Moberley Bay has received either untreated or primary-treated effluent. The mill began operation in 1948 and installed two primary treatment clarifiers during expansion in 1978. This work was completed prior to the installation of a secondary treatment aeration lagoon (September, 1989). Moberley Bay receives no other industrial or municipal effluents, making it an ideal site for studying the impact of BKME on fish populations. White sucker from Jackfish Bay spawn in tributaries to Jackfish Lake (Figure 1), the only suitable spawning streams within the Jackfish Bay drainage basin. Fish were captured from Sawmill Creek, an uncontaminated stream flowing into Jackfish Lake. The duration of residency in clean water prior to spawning is unknown and probably varies with weather conditions prior to and during the spawning season.

For comparison, white sucker were collected from a spawning run in the Little Gravel River, a tributary to Mountain Bay, Lake Superior (48°56'N, 87°50'W) (Figure 1). This reference site has been used previously to monitor impacts of BKME on Jackfish Bay fish populations (McMaster et al., 1991; Munkittrick et al., 1991a; Smith et al., 1991) as it receives no industrial effluent or significant domestic effluent input.

Sampling Procedures

Ripe fish were captured in overnight hoop net sets in both streams during mid-May, 1989. Fish were classified as ripe if they expelled gametes when light pressure was applied to their abdomen. Female fish were separated from males and were held in aerated holding tanks

of stream water prior to sampling. Male fish were transferred to holding nets and left in the streams until immediately before sampling. Females were sampled between 1030 and 1400 h and male fish from 1500 to 1830 h.

White sucker were rendered unconscious by a sharp blow to the head. Fork length (mm) and total weight (g) were determined, and each fish was dried with paper towels. Fish were aged by counting annuli on dried and cleaned opercula. Eggs were expressed into polyethylene bags using gentle pressure on the abdominal region. Male gametes were collected by inserting a 10 mL borosilicate pipette into the vent and applying gentle pressure to the abdominal region. The volume of milt expressed was measured to the nearest 0.5 mL. The milt was stored on ice in 100 mL polyethylene cups; subsamples were collected in unheparinized hematocrit tubes. Spermatocrit (percent packed sperm cell volume) was determined after centrifugation at maximum speed on an International Clinical Centrifuge (Model CL) with a hematocrit head for 7 min.

Spermatozoan motility was assessed by observing a small drop of fresh milt, diluted with fresh Sawmill creek water, in a AO Bright-Line hemacytometer under a light microscope at 100x magnification. Motility was evaluated on a subjective scale from 1 to 5, with 1 representing low motility and 5 representing high motility (Table 1) (Modified from Munkittrick and Moccia, 1987). The remaining milt was centrifuged at maximum speed for 7 min on an International Clinical Centrifuge (Model CL). The plasma was than decanted and stored at -20°C prior to chemical analysis. Samples were analyzed by flame atomic absorption spectrophotometry for sodium and potassium concentrations, on a Buchler-Cotlove chlorometer for chloride concentration and on a 5100B Vapor Pressure Osmometer for osmolality.

Fertilizations

Within and between site fertilizations were conducted to determine if the reproductive performance as measured by fertilization rate, embryo survival, and development, of either

males or females differed between the two sites. Male and female spawning white sucker were captured in overnight hoop net sets at both sites on May 22, 1989. Fish from the Little Gravel River were transported to Sawmill Creek in aerated holding tanks filled with Little Gravel River water. Females were held in these tanks prior to sampling between 1230 and 1400 h. Males were transferred to nets in Sawmill creek and sampled between 1430 and 1600 h. Sampling of fish was alternated between sites to prevent any bias due to sampling time. These fish were sampled similar to other spawning fish, except the gametes from both sexes were collected into 100 mL polyethylene cups and stored on ice prior to fertilization.

All fertilizations were performed within 5 h of gamete collection. Munkittrick and Dixon (1988) have shown that a time delay of 4 h would not cause any impact on fertilization rates in white sucker. Equal volumes of eggs were pooled from five females from each site. Milt from each of ten individual males (5 from each site) was used to fertilize 3 replicates of approximately 100 eggs from each of these pooled groups of eggs. Similarly, equal volumes of milt from the same 5 males from each site were pooled and 3 replicates of approximately 100 eggs from each individual female were fertilized with sperm from each of these pooled samples.

Each sample of approximately 100 eggs was placed into a plastic weigh boat. Five drops of milt were added, the eggs and milt were mixed with a plastic applicator stick for 5 s. They were allowed to sit for 5 min prior to the addition of 10 mL of Sawmill Creek water to initiate the water hardening/activation process. After 1 h of water hardening, the eggs were transferred to incubators (100 mL polyethylene beakers, modified by replacing the bottom with #656 Nytex screening). The incubators were placed into 36 L coolers containing 12°C water from Sawmill Creek and transported to the laboratory 4 d later. The water temperature was maintained at 11 to 12°C during this period by adding ice as required, and water was circulated by removing the lids of the incubators and flushing water over the eggs twice daily.

At the laboratory, the incubators were transferred to 64 L tanks supplied with 12°C well water at a flow rate of 1 L min^{-1} and incubated until hatching. Hardness, alkalinity and pH were measured weekly from randomly selected tanks. Mean (SE, n) characteristics of the water

were: total hardness, 344 (8.0, 8) mg L^{-1} as $CaCO_3$; Ca hardness, 244 (9.0, 8) mg L^{-1} as $CaCO_3$; Mg hardness, 100 (3.0, 8) mg L^{-1} as $CaCO_3$; alkalinity, 266 (6.0, 8) mg L^{-1} as $CaCO_3$; and pH, 7.3 (0.1, 8). Photoperiod was 16 h light: 8 h dark, with 0.5 h of gradual dawn and dusk included in the light portion. The eggs were treated every second day prior to hatch with malachite green, and fertilization estimates were performed at 6 d post fertilization. Dead eggs were removed daily. Deformity estimates were performed at hatch. The timing of hatch was variable, and was arbitrarily defined as the day on which 50 % of the eggs or larvae showed dark eye pigmentation (Munkittrick and Dixon, 1988; 1989). Deformities were categorized as either C-shaped, L-shaped, barbell, yolk at the posterior of larvae or a lump of yolk other than at the posterior.

Larvae were checked daily for developmental changes and samples were preserved in 10% buffered formalin 1, 7, 11, and 24 d after hatch. After preservation, the length (± 0.5 mm) and total weight (± 1.0 mg) of the larvae were determined. Leslie and Moore (1986) concluded that white sucker larvae did not show significant changes in length after preservation in formalin. After the total weight was determined, the yolk reserves were carefully dissected away and the larvae were reweighed (± 1.0 mg) to determine the relative contribution of the yolk to the total weight.

At 5 d post-hatch the larvae were separated into two groups. One group of 25 larvae from each replicate was separated into a new incubator; this group received no food, and the number of dead larvae removed daily was recorded. The remaining larvae were followed for developmental and behaviourial changes, and the initiation of exogenous feeding. Developmental changes included first swimming, bile in the gall bladder and gill circulation complete, mouth opening and closing, pectoral fins moving and blood entering the gill arches, swim bladder inflation, first feces, and yolk absorption complete. Fifty larvae were removed from this group on 21 d posthatch and frozen immediately in liquid nitrogen for analysis of mixed-function oxygenase (MFO) activity.

Mixed Function Oxygenase Determination

For the measurement of ethoxyresorufin-o-deethylase (EROD) activity, 50 whole larvae were thawed on ice and weighed (± 0.01 g). Samples were homogenized on ice in a hand-held homogenizer in 1 mL of HEPES-KCl (pH 7.5, 0.15 M KCL, 0.02 M HEPES) homogenization buffer. The homogenates were spun at 14,000 x g for 15 min at 4°C and the post-mitochondrial supernatant (PMS) was drawn off with a pasteur pipette. Samples were frozen at -80°C in 2.0 mL cryovials for use in the EROD assay. EROD activity determinations were completed following Munkittrick et al. (1991b), using ethoxyresorufin (ER) as the substrate, modified as outlined below.

For EROD determinations, PMS samples were thawed on ice prior to the assay. The reaction mixture contained, in this order, 1250 µL of 0.1 M HEPES buffer (pH 7.8), 10 µL of magnesium sulphate (0.154 mg mL^{-1}), 50 µL BSA (40 mg mL^{-1}), 30 µL reduced nicotinamide adenine dinucleotide phosphate (NADPH; 55 mg mL^{-1}), 500 µL of PMS and 20 µL of 7-ER (0.022 mg mL^{-1}) in 13 x 100 mm borosilicate glass tubes. Analyses were conducted at 25°C, and the reaction was stopped after 20 min by adding 3 mL of methanol. Blanks were prepared by adding 3 mL of methanol to the tubes prior to the addition of 20 µL 7-ER. The samples were centrifuged at 8000 x g for 5 min to pellet the precipitated protein. The supernatant was transferred to polycarbonate cuvettes and analyzed in a Perkin Elmer LS50 spectrofluorometer, with an excitation wavelength of 530 nm and an emission wavelength of 585 nm. The excitation slit width was 2.5 nm and the emission slit width was 20 nm. Results were converted to pmol mg^{-1} protein min^{-1} using a standard curve derived against increasing concentrations of resorufin.

Statistical Analysis

Length, weight, seminal plasma constituents, and milt volume differences between sites were compared using one-way analysis of variance (ANOVA). Mann-Whitney U-tests were used for age, sperm motility, deformity rate, and MFO comparisons between sites. Fertilization and deformity data were transformed with an arcsine square root function prior to analysis.

Three-way ANOVA (fertilization technique [pooled or individual] by female site by male site) was used to compare fertilization success and survival of the larvae (LT50s). One-way ANOVA with repeated measures was used to detect changes in growth, total weight, body weight and yolk absorption of larvae over time between sites.

RESULTS

The male fish used for examination of milt characteristics from Jackfish Bay (Sawmill Creek) were older than those from Mountain Bay (Little Gravel River)($p=0.005$). There was no difference between sites in milt volume ($p= 0.98$) or spermatocrit ($p=0.47$) but the motility of spermatozoa was higher at the reference site ($p<0.05$) (Table 2). Seminal plasma constituents could only be measured in males which released ≥2.0 mL of milt. Analysis indicated no differences between sites in male white sucker seminal plasma constituents, including sodium, potassium, chloride, and osmolality ($p>0.40$) (Table 3, Figure 2).

The age of the fish used for fertilization tests did not differ between sites for either males or females ($p>0.35$), and there was no difference in the weight or length of these fish ($p>0.30$). Jackfish Bay female gametes showed increased fertilization success compared to their Mountain Bay counterparts in all crosses performed ($p=0.035$, Table 4). There was no effect of capture site (Jackfish Bay or Mountain Bay), on the performance of male white sucker in the fertilization tests ($p=0.59$). The fertilization technique, whether it be pooled females crossed by individual males or individual females crossed by pooled males, had no effect on fertilization success ($p=0.07$).

The date of hatch was the same for both sites (14 days post-fertilization). Examination of the larvae at hatch found that there was no difference in the deformity rate of the larvae from eggs of BKME-exposed females relative to those at the reference site ($p=0.75$) (Table 5). The most common deformity, found at both sites, was a "c"-shaped curvature of the body where the larvae appeared to have hatched prematurely. The incidence of this deformity ranged from 63 to 89 percent of the total number of deformities found in eggs from any

individual female.

Both Jackfish Bay and Mountain Bay larvae developed at rates comparable to published developmental times for white sucker (McElman and Balon, 1980). Jackfish Bay larvae reached crucial development stages at times comparable to those for Mountain Bay larvae; developmental rates were nearly identical (Table 6). There was no apparent difference in larval behaviour between sites. There was no effect of capture site (Jackfish Bay or Mountain Bay), in either males ($p=0.11$) or females ($p=0.10$), and no effect of fertilization technique (pooled or individual fish) ($p=0.19$) on the survival of white sucker larvae. The mean survival time of unfed larvae from all crosses was 46 d post-fertilization.

At 1 d posthatch, there was no difference in either total length or total weight of larvae from Jackfish Bay compared to those from Mountain Bay ($p=0.053$ and $p=0.93$ respectively) (Table 7). There was however, a difference in length by 24 d, when larvae from Jackfish Bay were shorter than the reference larvae ($p=0.018$, Figure 3). From 1 to 24 d posthatch, there was a significant effect of age on the total length and total weight of larvae from both sites ($p<0.001$), and significant interactions between site and age for both length and total weight ($p=0.017$, $p=0.003$, respectively), indicating different growth rates. Although there was no difference in body weight at 1 day posthatch, there was a significant interaction between site and age ($p=0.038$), as Jackfish Bay larvae exhibited a slower increase in body weight, but used their yolk reserves at similiar rates to those larvae from the reference site ($p=0.50$, Table 7).

Mixed-function oxygenase activity in 21 d posthatch larvae was not different between the two sites ($p=0.347$). At this stage of development, larvae should have developed and functioning livers (McElman and Balon, 1980), as was indicated by the presence of dark green bile in the larvae.

DISCUSSION

Impacts of bleached kraft pulp mill effluent (BKME) on the adult white sucker at Jackfish Bay exist. These fish exhibit increased mixed-function oxygenase (MFO) activity, decreased plasma steroid hormone levels, decreased gonadal growth, and an increased age to maturation (McMaster et al., 1991; Munkittrick et al., 1991a).

One measure of reproductive performance is the fertility of male and female gametes. In this study, neither the source of the males nor the fertilization technique used had any effect on fertilization success; however, Jackfish Bay female eggs showed an increase in fertilization success compared to eggs from Mountain Bay. The fertilization percentages achieved were extremely high compared to similar white sucker fertilization tests (Munkittrick and Dixon, 1989), indicating no impact of prior BKME exposure to the adults on fertilization success. Ankley et al. (1989) attempted to correlate MFO activity (EROD) in females with fertilization success in chinook salmon (*Oncorhynchus tschawytscha*) from Lake Michigan. These fish also migrate to clean water to spawn, and no effect on fertilization success was found. Unlike these fish, starry flounder don't migrate to spawn, and Spies et al. (1985) found decreased fertilization success in fish collected from contaminated areas. It is unknown if these correlations are related to depuration, or whether the differences were related to impacts of polynuclear aromatic hydrocarbons (PAHs) (Spies et al., 1985) versus polychlorinated biphenyls (PCBs) (Ankley et al., 1989), versus the BKME studied here.

The older age of Jackfish Bay males corresponds to data collected for prespawning, and spawning male and female fish in 1989 (McMaster et al., 1991) and for both sexes from the preliminary study in 1988 (Munkittrick et al., 1991a); white sucker in Jackfish Bay are older, shorter and lighter than those from the reference site. Milt from BKME-exposed white sucker was similar to milt from reference males in volume released, spermatocrit values, and seminal plasma constituents including sodium, potassium, chloride, and osmolality. Although spermatozoa showed reduced motility relative to those from Mountain Bay males, this had no effect on fertilization performance. Seminal plasma constituents are important in motility, as Munkittrick and Moccia (1987) found that high motility was correlated with high seminal

plasma concentrations of Na^+, K^+, or Cl^-, and that high ionic concentration was inversely related to volume. Lower motility of Jackfish Bay spermatozoa does not appear to be a result of lower seminal plasma ion concentrations since levels were similar between sites. The reduction in motility could become important if critical spermatozoa : egg ratios were reached, as Moccia and Munkittrick (1987) found highly significant correlations between subjective motility and fertilization estimates when critical ratios were present. When excess sperm was used, as occurred in this study, low motility samples yielded fertilization rates comparable to those rates yielded by high motility samples.

Although Jackfish Bay males have been shown to have reduced gonadal weights in August, testes in spawning fish are similar in size and no correlation to milt volume was found. Freeman and Idler (1975) found that PCB treatment to brook trout (*Salvelinus fontinalis*) resulted in regressed testes, reduced androgen levels and reduced production of the spermatic fluid.

There was no effect of capture site on the time to hatch or in hatching success of larvae. The hatching process whereby the protective egg membrane disappears and the gills of the sac fry begin to develop functionally may alter the path of exposure, and therefore sensitivity, to environmental pollutants (Van Leeuwen et al., 1985). This may be important for species which spawn in contaminated areas, but is not a factor for Jackfish Bay white sucker eggs. Eggs from PCB treated females fertilized by PCB treated males resulted in only 78% hatch compared to 100% in control fish (Freeman and Idler, 1975). When control eggs were incubated in PCB water, less than 1% hatched and when control larvae were held in PCB water, the young lived only a few days. This suggests that the eggs and the young are much more sensitive to PCB than the adult fish.

At the time of hatch, no difference in the deformity rates were found. These rates were however, quite high compared to other studies on white sucker, as deformities ranged from 2.2 to 6.6 % in fish from a metal contaminated lake and 1.5 % for the reference population (Munkittrick and Dixon, 1989). The major type of deformity at both sites in this study was a "c"-shaped curvature of the body accompanied by premature hatch. It represented 74 to 83

% of the deformities present. This deformity was also high at the metal contaminated lake (87%), but was not the dominant deformity found in Lake Ontario white sucker larvae (29%) (Munkittrick et al., 1989). The cause of this specific deformity is unknown, but it may reflect premature hatching of the embryo, as the body is "c-shaped" within the egg shell prior to hatch.

Eggs in Jackfish Bay females do not increase in size with age, as was noted at the reference site (McMaster et al., 1991; Munkittrick et al., 1991a). Although eggs were smaller at the BKME site, there was no difference in larval total weight or length at time of hatch. There was however, an impact on growth rates; Jackfish Bay larvae were significantly shorter by 24 d posthatch. Work on the effect of egg size on early growth and survival of rainbow trout (*Oncorhynchus mykiss*) has indicated that larger eggs, from older females, produced larger larvae at hatch, a correlation that was lost 4 wk after first feeding. Egg size had no effect on survival rates to eye-up, hatch, or swim-up (Springate and Bromage, 1985). Lehtinen (1989) found that different bleaching processes had different effects on the survival and growth of sticklebacks (*Gasterosteus aculeatus*). Conventional bleaching with chlorine caused acute mortality of larvae, while other processes showed reduced growth of larvae. The bleaching process at Terrace Bay is a conventional bleaching process with some addition of chlorine dioxide.

The decreased growth of larvae may be quite significant, as adult fish from Jackfish Bay are significantly shorter at the age of maturation compared to two reference populations (McMaster et al., 1991; Munkittrick et al., 1991a). Although they seem to grow at similar rates after maturation, BKME fish are always shorter at a specific age. Although we found no increase in the yolk absorption rates of larvae from the BKME site in clean water, the efficiency of the conversion of this energy to growth seems to be impaired. Other studies have found that alevins exposed to BKME consumed their yolks faster than the controls (Ruoppa and Nakari, 1988). This would have no impact on white sucker larvae, as they migrate to clean water to spawn, but for a species such as whitefish that are present in high numbers in the effluent plume and spawn in the lake, this may have a dramatic impact on survival of their young.

The lack of any significant differences in developmental rates or in larval survival, suggests that female white sucker are not predisposing their young to lethal levels of contaminants through the deposition of lipophilic xenobiotics into the yolk of their eggs. Survival of unfed larvae corresponds well with that of reference larvae reported by Munkittrick and Dixon (1988). This differs from work on BKME-exposed pike (*Esox lucius*) whose eggs showed significantly increased mortality compared to those from unexposed areas when raised in clean water (Tana and Nikunen, 1985). Accumulation of toxicants followed by metabolism of triglycerides and proteins during the process of yolk absorption increases the concentration, and possibly also the accessibility of pollutants and thereby toxicity (Van Leeuwen et al., 1985). The yolk absorption process is believed to be the major factor in the re-distribution of chemicals causing death among pike exposed to TCDD (Helder, 1980).

Although EROD activity was detectable in 21 d posthatch larvae, no difference between sites existed. Adult white sucker from Jackfish Bay have increased MFO activity throughout the year compared to the reference site (McMaster et al., 1991). However, this induction is reduced or eliminated during the spawning period. It is not clear whether this reduction is due to normal seasonal changes or to migration of the fish from the BKME-contaminated site to an uncontaminated site (Sawmill Creek) for spawning. Whether enzyme activity is transferred from the female to the progeny along with lipophilic contaminants is unknown. However, this may be beneficial to larvae that are hatched in contaminated areas increasing their tolerance to the pollutant (Stegeman and Kloepper-Sams, 1987).

The development of a functional digestive system (including detoxification mechanisms) during ontogenesis, may alter the toxicological response in larval fish (Van Leeuwen et al., 1985). Embryonic tissues can be particularly sensitive to damage by xenobiotic metabolizing enzymes, however relatively little is known about the capacity of these stages to biotransform xenobiotics (Binder and Lech, 1984). MFO activity has been found in embryos and swim-up fry of Lake Michigan lake trout (*Salvelinus namaycush*). This activity had no effect on survival of larvae reared under hatchery conditions and activity was equal to controls after 210 d in clean water. Clearly induction of cytochrome P-450 systems is not grossly detrimental to normal development. Binder and Stegeman (1984) found aryl hydrocarbon

hydroxylase (AHH) activity in all embryos starting from 4 d post-fertilization. This activity occurred prior to liver development and therefore had to be that of extrahepatic tissues. Increase in AHH activity at hatch may be a general feature of the ontogeny of cytochrome P-450 systems in fish. It has been shown that relatively low levels of this MFO activity are present during the most active period of organogenesis prior to hatching (Binder and Stegeman 1984). Damage from activated metabolites during this sensitive phase of development could severely reduce the viability of the developing organisms. After hatching the need to metabolize and eliminate foreign compounds is likely to become important (Binder and Stegeman, 1984).

Whether fertilization success of these white sucker populations would be similar in the wild is unknown. These fish do have significantly reduced plasma steroid levels during the spawning period and male fish showed significantly reduced secondary sexual characteristics (nuptial tubercles) compared to males from the Mountain Bay site (McMaster et al., 1991). Lake Michigan lake trout do not to reproduce successfully in the wild. However, their eggs and larvae survived equally well under hatchery conditions (Binder and Lech, 1984). This suggests that no direct link between larval survival in the lab and their survival in the wild exists. Depressed steroid levels may interfere in normal spawning behaviour of both male and female white sucker at the BKME-exposed site. Fleming and Gross (1991) found that lower steroid levels in hatchery reared coho salmon (*Onchorynchus kisutch*) corresponded to reduced dominant spawning behaviour and spawning success relative to the wild population. Whether such changes exist at Jackfish Bay remains to be seen.

CONCLUSIONS

White sucker exposed to bleached kraft mill effluent exhibit a number of physiological impacts including decreased egg and gonadal size, and decreased plasma steroid levels. These fish however, still produce viable gametes and these gametes are of equal or greater fertility then those from the reference site. Although spermatozoan had reduced motility, this has no impact on the males fertilization potential. Eggs hatch and larvae survive equally well, but

they do show decreased growth rates compared to reference larvae. Although MFO activity is present in 21 d larvae, no differences between sites exist.

ACKNOWLEDGEMENTS

Funding for this study was provided by the Research Advisory Council, Ontario Ministry of Environment. The assistance of Ms. Linda Melnyk-Ferguson Ontario Ministry of Natural Resources (OMNR), Terrace Bay, Ontario and Mr. Lorne Townes OMNR, Nipigon, Ontario are gratefully acknowledged. This report was prepared for the Ontario Ministry of Environment (MOE), as part of a MOE-funded project related to Jackfish Bay. The views and ideas expressed in this report are those of the authors and do not necessarily reflect the views and policies of the Ontario provincial government, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. Special thanks to Allan Arthur for his help in the laboratory portion of this work and to the Water Quality Lab at the University of Waterloo for providing chloride analysis.

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Table 1

Subjective rating scale for motility of spermatozoa from white sucker collected during the spawning period (adapted from Munkittrick and Moccia, 1987).

Rating	Description
1	Very poor motility - 0 to 20% of the spermatozoa moving slowly in the field of view.
2	Poor motility - 20 to 40% of the spermatozoa moving rapidly in the field of view.
3	Good motility - 40 to 60% of the spermatozoa moving rapidly in the field of view.
4	Very good motility - 60 to 80% of the spermatozoa moving very rapidly in the field of view.
5	Excellent motility - 80 to 100% of the spermatozoa moving very rapidly in the field of view.

Table 2

Milt characteristics of all males collected during the spawning run from the BKME exposed (Jackfish Bay) and reference (Mountain Bay) sites.

Site	Volume (mL)	Spermatocrit (%)	Motility (Score[1])
Jackfish	4.79±0.81 (22)	78.8±4.4 (22)	3.0±0.3 (21)*
Mountain	4.77±0.62 (31)	82.7±3.3 (31)	3.7±0.2 (31)

* different from control, $p<0.05$

[1] scored from 1 to 5, with 1 representing low motility

Table 3

Milt characteristics and seminal plasma constituents of male white sucker from Jackfish Bay (BKME) and Mountain Bay (reference) spawning runs. Values are from males that produced 2.0 mL or more of milt and are given as mean ± s.e. and (n) if different.

Site	N	Volume (mL)	Spermatocrit (%)	Motility (Score[1])	Na (mmol L^{-1})	K (mmol L^{-1})	Cl (mmol L^{-1})	Osmolality (mOs kg^{-1})
Jackfish Bay	16	6.22 ± 0.98 (15)	74.6 ± 5.3	3.1 ± 0.3*	38.7 ± 1.3	9.6 ± 0.5	54.9 ± 3.9 (14)	206 ± 8.0 (14)
MountainBay	22	6.12 ± 0.68	81.4 ± 4.4	4.0 ± 0.2	37.4 ± 1.3	9.6 ± 0.4	53.6 ± 4.5 (15)	200 ± 7.5 (21)

* different from control, $p<0.05$

[1] scored from 1 to 5, with 1 representing low motility

Table 4

Fertilization percentage of eggs from individual or pooled females from Jackfish Bay (BKME) and Mountain Bay (reference) fertilized by individual or pooled males from both sites. Each cross represents approximately 1500 eggs from five individual males and females from each site. Values are given as mean ± s.e..

	Males[1]		Females[2]	
Site	Mountain	Jackfish	Mountain	Jackfish
Mountain	96.3 ± 1.1	97.2 ± 1.0	94.4 ± 1.9	97.6 ± 0.8
Jackfish	98.0 ± 0.4	97.9 ± 0.7	94.5 ± 0.9	95.9 ± 1.5

[1] individual males to pooled females

[2] individual females to pooled males

Table 5

Frequency of larval deformities (%) and percentage of deformed white sucker larvae from the BKME exposed (Jackfish Bay) and reference (Mountain Bay) sites.

		Type of deformity				
Site	Deformity %	C-Shape	Barbell	Yolk at Posterior	Lump of Yolk	L-Shape
Jackfish Bay	10.2	83.1	6.4	1.1	7.5	1.8
Mountain Bay	8.6	73.8	10.0	5.6	10.6	0

Table 6

Age at which white sucker larvae from the reference (Mountain Bay) and BKME contaiminated (Jackfish Bay) sites reached crucial developmental stages (Munkittrick and Dixon, 1989a,b; modified from McElman and Balon, 1980) at 12°C. Times are given in days post-hatch and daily temperature units (DTU).

Critical Stage		Jackfish Bay			Mountain Bay		
		Age (d)	DTU (°d)	Incid (%)	Age (d)	DTU (°d)	Incid (%)
I.	First swimming (<2.5cm)	5	60		5	60	
II.	Bile in gall bladder, gill circulation complete	11	132		11	132	
III.	Mouth opening and closing, pectoral fins moving and blood entering gill arches	14	168		14	168	
IV.	Swim bladder inflated	17	204	35	17	204	30
		20	240	60	20	240	60
		24	288	100	24	288	100
V.	First feeding, first feces	27	324	25	27	324	30
		30	360	100	30	360	100
VI.	Yolk absorption complete	24	288	4	24	288	16
		30	360	100	30	360	100

Table 7

Total wet weight, body weight (total weight-yolk weight), percent yolk and total length of white sucker larvae from the BKME exposed site (Jackfish) and the reference site (Mountain) at different ages post-hatch. Values are given as means ± s.e.(n).

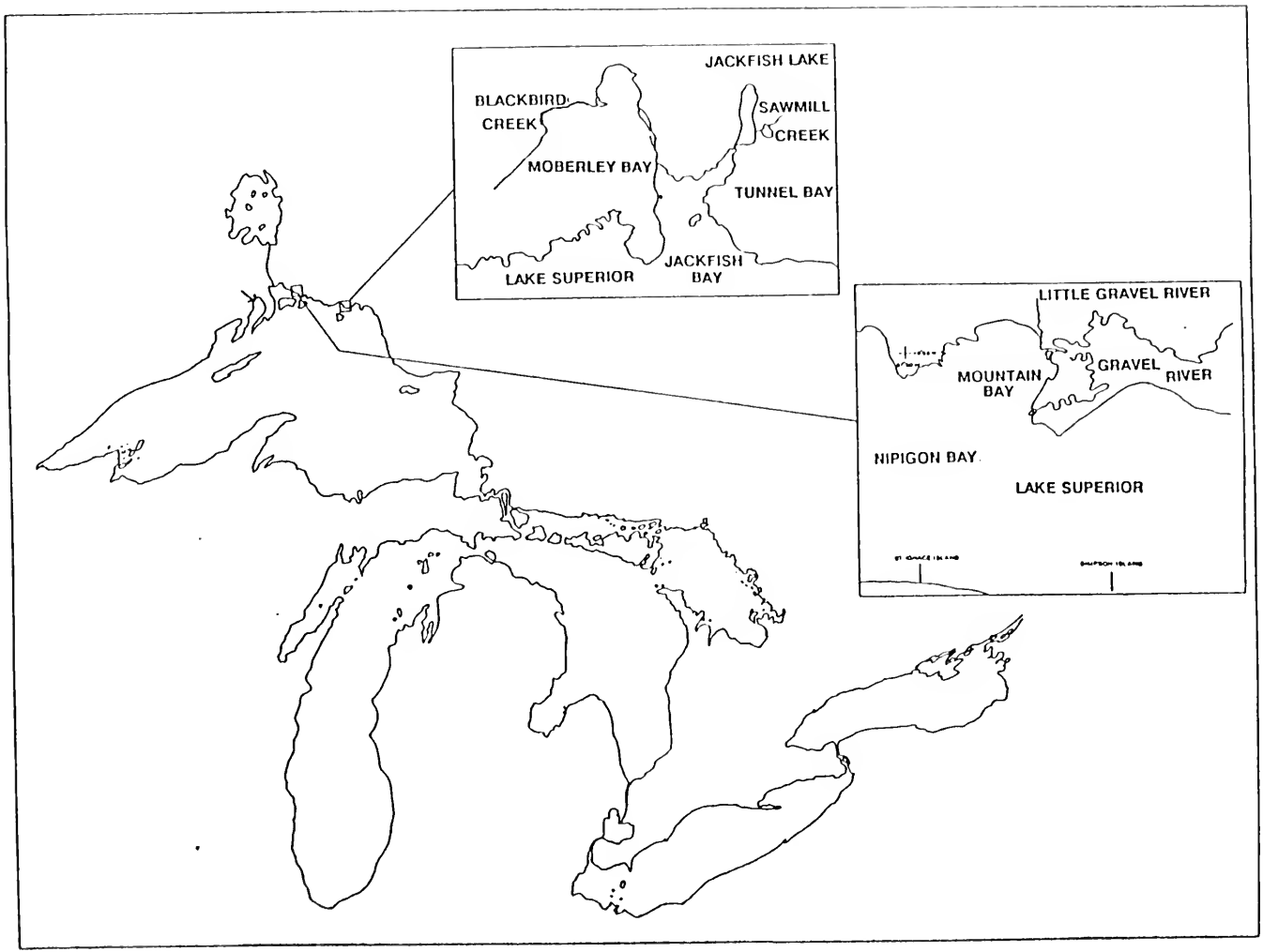
Site	Age (d)	Total Weight (mg)	Body Weight (mg)	Percent Yolk (%)	Total Length (cm)
Jackfish Bay	1	6.919±0.363 (50)	2.237±0.140 (50)	67.5±2.4 (50)	0.968±0.019 (50)
	7	7.070±0.515 (50)	3.189±0.086 (50)	54.6±4.1 (50)	1.152±0.035 (50)
	11	7.750±0.229 (50)	4.521±0.211 (50)	41.6±3.9 (50)	1.275±0.013 (50)
	24	7.994±0.573 (50)	6.816±0.355 (50)	14.3±6.5 (50)	1.415±0.021 (50)*
Mountain Bay	1	6.960±0.862 (50)	2.187±0.291 (50)	68.5±2.4 (50)	1.001±0.022 (50)
	7	7.185±0.748 (50)	3.036±0.542 (50)	57.9±4.3 (50)	1.166±0.052 (50)
	11	7.676±0.768 (50)	4.558±0.565 (50)	40.7±3.5 (50)	1.321±0.075 (50)
	24	8.705±0.747 (50)	7.342±0.701 (50)	15.6±6.1 (50)	1.504±0.056 (50)

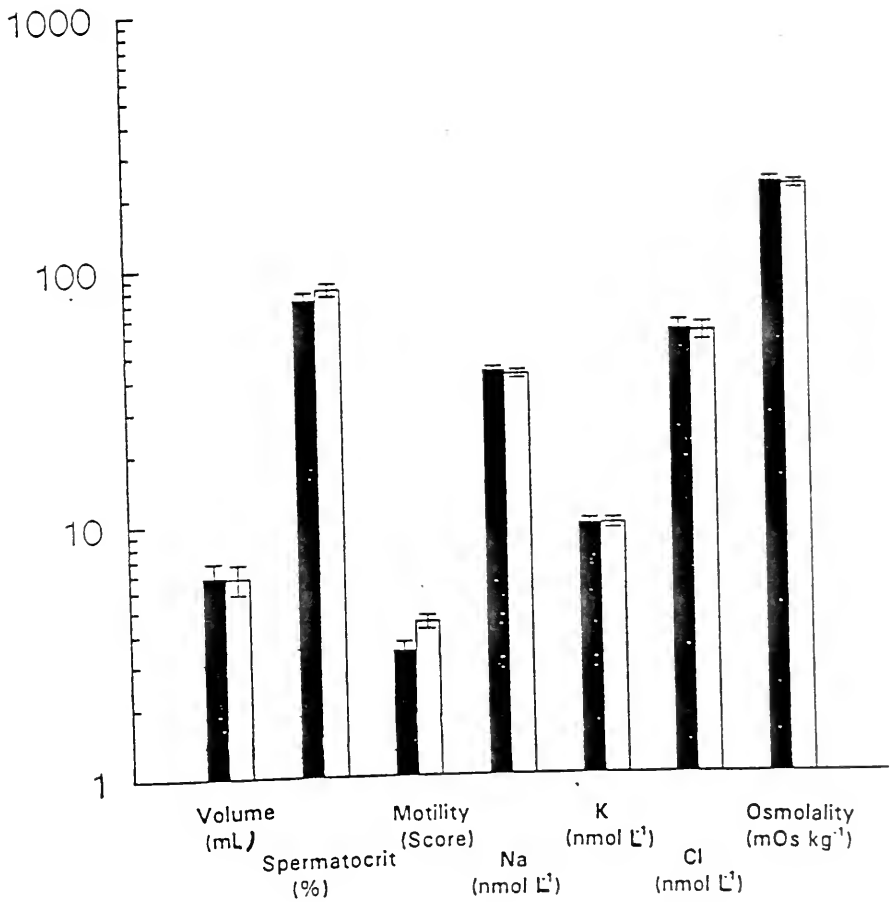
Legend to Figures

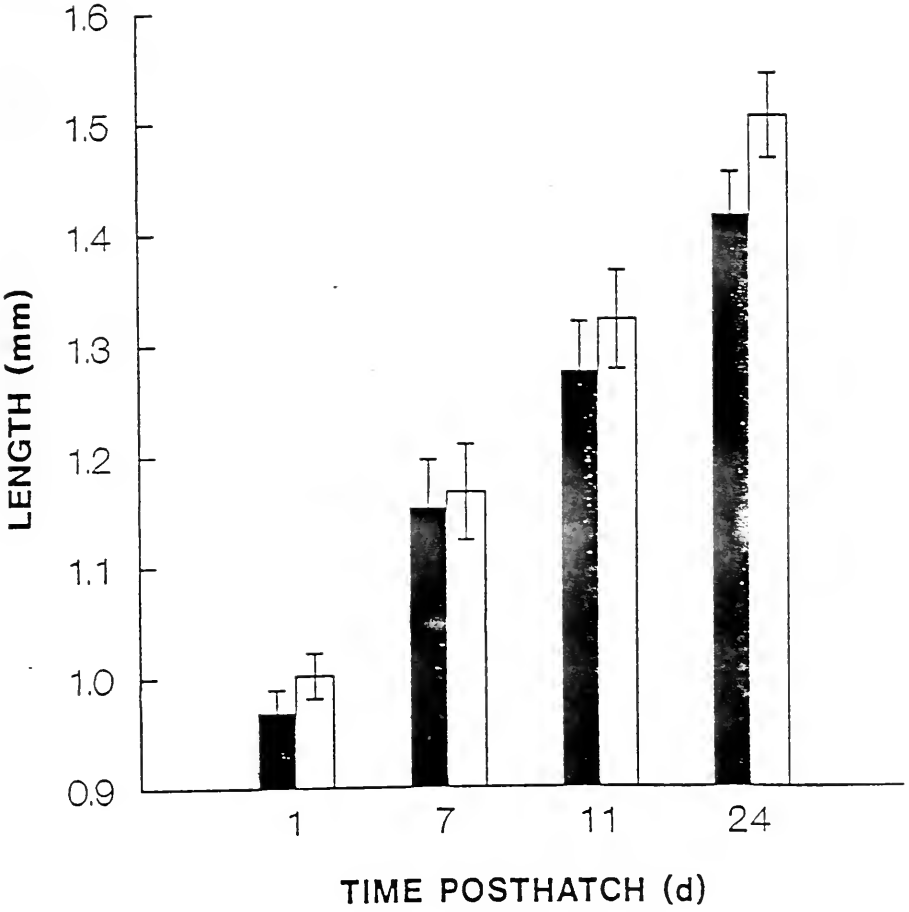
Figure 1 The Study Site. Effluent from the bleached kraft mill at Terrace Bay, Ontario reaches Lake Superior at Jackfish Bay, with Mountain Bay serving as reference site.

Figure 2 Milt characteristics; volume, spermatocrit, motility, sodium, potassium, chloride, and osmolality, from male white sucker exposed to BKME (Jackfish Bay) and those from a reference (Mountain Bay) location.

Figure 3 Growth of white sucker larvae from Jackfish Bay (BKME exposed; solid bars) and Mountain Bay (reference; empty bars) when reared under laboratory conditions in clean water.


JACKFISH LAKE
BLACKBIRD CREEK
SAWMILL CREEK
MOBERLEY BAY
TUNNEL BAY
LAKE SUPERIOR
JACKFISH BAY
LITTLE GRAVEL RIVER
MOUNTAIN BAY
GRAVEL RIVER
NIPIGON BAY
LAKE SUPERIOR


1000
100
10
1
Volume (mL)
Spermatocrit (%)
Motility (Score)
Na (nmol L-1)
K (nmol L-1)
Cl (nmol L-1)
Osmolality (mOs kg-1)
MILT PARAMETERS


1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
LENGTH (mm)
1
7
11
24
TIME POSTHATCH (d)

External lesions and changes in maturity, MFO activity and plasma sex steroid levels of lake whitefish exposed to bleached kraft mill effluent (BKME).

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To submit to Can. J. Fish. Aquat. Sci.

Abstract

Munkittrick, K.R., M.E. McMaster, C.B. Portt, G.J. Van Der Kraak, I.R. Smith and D.G. Dixon. 1991. External lesions and changes in maturity, MFO activity and plasma sex steroid levels of lake whitefish exposed to bleached kraft mill effluent (BKME). Can. J. Fish. Aquat. Sci. 00 : 0000-0000.

Lake whitefish (Coregonus clupeaformis) exposed to primary treated bleached kraft pulp mill effluent (BKME) had reduced gonadal development and increased liver size relative to two reference populations. These results parallel our previous work on the white sucker (Catostomus commersoni) exposed to BKME at the same Lake Superior site. More detailed studies conducted in 1990, after the installation of a secondary treatment aeration lagoon at the pulp mill, found that lake whitefish exhibited reduced gonad sizes, delayed age to maturation, decreased levels of the plasma sex steroids testosterone and 17β-estradiol, and elevated hepatic mixed function oxygenase (MFO) activity. Liver size was smaller following operation of the secondary treatment system. More than 20% of the whitefish collected at the BKME site exhibited lateral, slash-like lesions which penetrated the body cavity. Histological examination revealed no evidence of an infectious etiology, and the wounds could not be accounted for by known causes. Similar lesions were found in 1991 near a second BKME discharge. The restriction of these lesions to two sites receiving BKME, and the correlation with other adverse impacts suggests that the BKME discharge may be the causative agent.

Introduction

There have been extensive descriptions of impacts of bleached kraft mill effluent (BKME) on fish populations in the Baltic Sea (reviewed in Sodergren 1989), but until recently there was relatively little information available from field studies in North America. Studies conducted at Jackfish Bay, Lake Superior, in 1988 (Munkittrick et al. 1991a) and 1989 (McMaster et al. 1991a,b) revealed impacts of BKME on white sucker (Catostomus commersoni) at both the physiological and population level. White sucker exposed to BKME exhibited delayed sexual maturity, smaller gonads, reduced egg size, reduced secondary sexual characteristics, increased liver size, increased condition factor and a smaller length at age than white sucker from comparable reference sites. White sucker exposed to BKME also exhibited reduced levels of plasma sex steroids (testosterone, 11-ketotestosterone, 17β-estradiol and 17α20β-dihydroxy-4-pregnen-3-one) and elevated levels of hepatic mixed function oxygenase (MFO) activity as measured by the metabolism of benzo(a)pyrene, diphenyloxazole and ethoxyresorufin.

A more recent study on the St. Maurice River found increased MFO activity and decreased testosterone levels in male white sucker collected downstream from a bleached kraft mill at La Tuque, Quebec (Hodson et al. 1991). Impacts of BKME on MFO activity have been reported in white sucker collected near other bleached kraft mills in Ontario (Servos et al. 1991; Smith et al. 1991). A fundamental question arising from these studies is whether similar responses to BKME are found in other teleost species. Elevated MFO activity has been reported for lake trout (Salvelinus namaycush; Munkittrick et al. 1991b) and juvenile chinook salmon (Oncorhynchus tshawytscha; Rogers et al. 1989) exposed to BKME, but these studies did not include the measurement of circulating sex steroids, and did not address impacts on reproduction.

Jackfish Bay receives the effluent from a 1200 tonne per day bleached kraft mill situated in Terrace Bay, Ontario. The pulp mill installed an aerated lagoon system at a cost of more than $20 M, which became operational in September, 1989. Up until the initiation

of secondary treatment, the effluent entering Jackfish Bay had received only primary treatment (or no treatment) for 40 years. This study had two main objectives, a) to examine reproductive development in lake whitefish (Coregonus clupeaformis), a commercially important Lake Superior species, and b) to compare the responses of fish collected during primary treatment (August 1989) with fish collected during the first year of secondary treatment (August 1990). Examination included organ size, growth rates, MFO activity and levels of plasma sex steroids.

Dioxins and furans, and dioxin-like compounds have been shown to induce MFO activity, and have been suggested as factors responsible for increasing MFO activity downstream of bleached kraft mills (Rodgers et al. 1989; Hodson et al. 1991). As well as their inducing properties, mammalian studies with dioxins have shown them to induce immunotoxicity (Poland and Knutson 1982). Andersson et al. (1988) reported increased frequency of external pathological fin alterations and reduced white blood cell counts in perch (Perca fluviatilis) exposed to BKME, indicating a suppressed immune response. Since elevated dioxin levels (Sherman et al. 1990), induced MFO activity (McMaster et al. 1991a; Munkittrick et al. 1991a) and reduced concentrations of white blood cells (Munkittrick, unpubl. data) have been found in white sucker at this site, a survey of organs for histological evidence of impacts was conducted on lake whitefish.

Methods

Effluent from the Terrace Bay mill reaches Jackfish Bay, Lake Superior through Blackbird Creek (Figure 1). Jackfish Bay has no residential or industrial development, and receives no other industrial waste. Lake whitefish were collected from Jackfish Bay using gill nets in the summer periods (August 12-13, 1989; August 12-14 and September 27-30, 1990). Lateral "slash-like" lesions were first noticed on a small number of fish collected in the effluent plume during August of 1989. As lake whitefish are rare in these shallow waters near the mouth of Blackbird Creek, collections during August, 1990, were made in deep

water (>28 m) near Little Nick rock (approximately 1.8 km off the mouth of Blackbird Creek; Figure 1). Reference collections were conducted in Mountain Bay (August 10-11 1989; August 9-11, 1990; latitude 48°56'N longitude 87°50'W) and Black Bay (August 20-21 1989; October 1-3, 1990; 48°38'N, 88°32'W). Complete descriptions of these study sites are found in Munkittrick et al. (1991a) and McMaster et al. (1991a).

Calculation of exposure levels to BKME at Jackfish Bay is difficult, due to increased surface temperatures associated with the effluent, the presence of a strong thermocline in summer months and the influence of winds on mixing and distribution of the surface water plume. Based on data collected in 1987 and 1988 (K. Sherman, Ontario Ministry of Environment, Toronto, ON M4V 1P5; unpubl. data), surface dilutions are estimated to be 5:1 at the sampling site and 15:1 at Little Nick rock. However, these dilution calculations may be misleading. Chemical concentrations have only been taken near the water surface, and the effluent plume is only present in the top 3 to 4 m of water; the fish examined have all been benthic feeders and were captured at depths of 6 to >28 m. Exposure would be better indicated by sediment concentrations of contaminants. Total organic carbon (TOC) values in the effluent prior to secondary treatment approximate 184-318 µg/mL (Environment Canada 1991) while sediment levels range from 400 µg/g at the mouth of Blackbird Creek, to 50-60 at the plume station, 35-40 at Little Nick and 30 at reference sites (Sherman, unpubl. data), corresponding to TOC levels of 5.4% at the plume station and 1.4% at Little Nick, relative to the mouth of Blackbird Creek.

During both years, fork length, total weight, liver weight and gonad weight were determined for each fish. The left operculum of each fish was removed and frozen. Opercula were later boiled, and the annuli counted to determine their age. In 1990, blood was collected from live whitefish via caudal puncture into 5.0 mL heparinized vacuutainers and placed on ice for 6 to 8 h. The plasma was collected after centrifugation for 7 min, frozen in liquid nitrogen and stored at -80°C prior to analysis. Testosterone and 17β-estradiol were measured by radioimmunoassay (RIA) in plasma following ether extraction. A description of the antisera used to measure testosterone was reported in Van Der

Kraak et al. (1984) and for 17β-estradiol in Van Der Kraak et al. (1990). Measurement of 17β-estradiol and testosterone followed the description of Van Der Kraak et al. (1984). All plasma samples were assayed in duplicate and interassay variability was less than 15% in both RIA systems.

Approximately 1 g of liver was placed in cryovials, frozen immediately in liquid nitrogen, and stored at -80°C pending analysis. In the laboratory, the samples were thawed on ice and analyzed for mixed function oxygenase (MFO) activity using ethoxyresorufin as the substrate via a modification of the ethoxyresorufin-o-deethylase (EROD) methods described in Rogers et al. (1989) and Muir et al. (1990).

External lesions were subjectively categorized on a scale of 1 to 7, with increasing numbers from 1 to 5 reflecting a progressive increase in the size and severity of the lesion, and with numbers greater than 5 reflecting progressive healing (Table 1).

Analysis of covariance (ANCOVA) was used within years to test for differences between sites in condition factor (CF) (slope of fork length versus adjusted body weight [total weight-gonad weight-liver weight]). Differences in slopes of the regressions indicate differences in rates of body weight gain with length, indicating a difference in CF between sites. Differences in gonad weight and liver weight between sites was also tested using ANCOVA analyses, with adjusted body weight as the covariate. These data were first analyzed for homogeneity of slopes, and if slopes were similar (>0.05), the data was then tested for differences between intercepts, which signifies differences between sites. In 1989, three sites were sampled (two reference, one contaminated). When intercept differences were present, direct contrasts between sites were completed to determine which sites were different. All analyses were completed using log transformed values. Only 5 whitefish were captured at the reference site in September, making analyses difficult between sites. Therefore, these analyses were completed only on August 1989-1990 data and not the September data, however the numbers are presented to show their seasonal changes. Kruskal-Wallis analysis was used to determine differences between

sites in age, hepatic EROD activities, and plasma testosterone and 17β-estradiol levels.

Results

Male whitefish were significantly younger at Jackfish Bay ($p=0.007$), although there was no difference for females ($p=0.28$). There were no differences in condition factor (CF) between Jackfish Bay and the reference sites; slopes of regressions on fork length versus adjusted body weight were not different between sites for either males or females in 1989 ($p = 0.97$ and $p = 0.82$ respectively) or 1990 ($p = 0.25$ and $p = 0.63$).

During 1989, both males and females were significantly shorter and lighter at the Mountain Bay reference site, and both reference sites exhibited smaller livers and larger gonads than Jackfish Bay, for both sexes (Table 2). ANCOVA analysis of liver weight versus adjusted body weight in 1989 indicated no differences in the slopes for either males or females ($p = 0.91$ and $p = 0.34$, respectively). However, intercepts were significantly different between sites for both males and females ($p<0.001$), indicating that liver weights were larger at the BKME site for both sexes, relative to both reference sites (Figure 2a). In 1990, this relationship was not evident, as intercepts were the same between sites for both males ($p = 0.08$) and females ($p = 0.13$), indicating similar liver sizes between sites (Figure 2b). This reduction in liver size is evident by the decline in the liversomatic index (LSI), as it dropped from 1.94 to 1.35 in males and from 2.41 to 1.52 in females (Table 2) at the Jackfish Bay site from 1989 to 1990, while values from the reference site remained relatively constant.

Fish from the Mountain Bay and Black Bay reference sites had larger gonads relative to the BKME site in August 1989, as ANCOVA analysis indicated similar slopes ($p>0.33$) but different intercepts for both males and females ($p<0.001$) (Figure 3). In 1990, similar trends were found as intercepts were different ($p<0.001$) between sites for both sexes,

however in male whitefish, the slopes of gonad weight versus adjusted body weight were different between sites ($p = 0.015$). In male whitefish, there was no difference in body weight between sites in August 1990, however testicular size at the BKME site was only 1/3 the size found at the reference site ($p<0.001$; Table 2). Gonadosomatic indices (GSIs) indicate no recovery of the gonads at the Jackfish Bay site in either males or females following secondary treatment in 1990 (Table 2).

All male whitefish collected from the reference sites were sexually mature; the smallest GSIs in these fish was approximately 1% (8 g in an 800 g fish; Figure 3a). More than 90% of male whitefish (33/36 fish) collected at Jackfish Bay in August were immature, exhibiting low GSIs (<1% body weight), compared to 2/41 male fish at the reference sites which exhibited low GSIs (5% of fish) (Figure 3a). This represents a marked delay in sexual maturation. Based on regressions of testes weight versus body weight, reference males would produce a 10 g gonad at an August body size of 742 g, relative to a Jackfish Bay male whitefish size of 1615 g. Since Jackfish Bay whitefish grow at the same rate as reference fish, this represents a delay in maturity from 6.0 to 10.4 y, based on a regression of age versus weight for Jackfish Bay male whitefish (age=0.005 weight + 2.28, n=24, r^2=0.77). The maximum age of whitefish found was 12 to 13 y.

Females followed a similar trend, but were not affected as dramatically (Figure 3b). All females from the reference sites were developing ovaries for the upcoming spawning season, and the smallest ovaries were approximately 20 g, representing a GSI of >2.5%. Almost 50% of the females from Jackfish Bay had GSIs below 2%, and showed no evidence of ovarian development for the fall spawning period. As an indicator of delayed maturity, females at the reference sites would achieve a 20 g gonad during August at a weight of 549 g, relative to 994 g at Jackfish Bay (Figure 3b), which corresponds to a delay in maturity from 5.5 to 7.8 y (age=0.005 weight + 2.79, n=23, r^2=0.85). However, mean fecundity estimates (August, 1990 only) for Jackfish Bay (mean, SE, n; 26728, 3010, 16) were higher than for Mountain Bay (14294, 1750, 14) ($p=0.002$). Although fecundity estimates were double at Jackfish Bay, mean gonadal weights were almost 50%

smaller (Table 2), suggesting that egg size was markedly reduced at Jackfish Bay.

Both BKME males and females had reduced plasma sex steroid levels in August 1990 (Figure 4). Testosterone levels in males from Jackfish Bay were 3.4-fold lower then those from the reference site ($p<0.001$), while female testosterone and 17β-estradiol levels were 3.2 ($p<0.001$) and 4.3-fold ($p<0.001$) lower than the levels in females from the reference site. MFO activity (EROD) from Jackfish Bay were increased 11.6-fold in females ($p=0.002$) and 5.2-fold in males ($p=0.007$) relative to the reference site (Figure 5).

Analyses for growth, gonadal and liver differences between sites in September were not completed as only 5 specimens were collected at the reference site in September, all of which were sexually immature (i.e. GSIs <1%)). This presumably reflects the proximity of collections to the spawning period and migration of mature adults to an unidentified spawning area.

Lesions

Lateral, elongated, "slash-like" lesions, which penetrated the body cavity were evident on more than 20.5% (24/117) of lake whitefish collected during August, 1990 (Figure 6); no such lesions were found at reference sites. The 24 fish with lesions had a total of 31 lesions (3 fish with 2 lesions, 2 fish with 3 lesions), of which 27 extended into the peritoneal cavity, or represented scars of wounds which had extended into the body cavity.

Of the 18 lesioned fish sampled in detail during August, 1990, there were no detectable effects of sex or size on lesion prevalence. Females constituted 56% of the samples, and the average length and weight were not significantly different from non-lesioned fish. The lesions were unilateral in 23 of 24 fish, and were not symmetrical on the single fish with both sides lesioned. There was an equal prevalence on the right (15 lesions) and left side of fish (16 lesions). All lesions were on the body cavity, with 11 occurring in the

pelvic region, 10 in the anterior region (below the lateral line), 6 in the posterior region and 4 lesions near the midline of the fish, extending to ventral areas (Figure 7).

Histological sections of gill, liver, spleen, heart, anterior and posterior kidney were examined from whitefish from Jackfish Bay (n=11) and Mountain Bay (n=14). Lesions in the gill, heart, spleen, anterior kidney and liver were minor, consistent with collection of wild fish and no differences were detected between sites. The most striking changes were associated with the muscular lesions found on Jackfish Bay whitefish. Several of these lesions were examined microscopically, and all featured necrotic, waterlogged muscle, with mild lymphocytic inflammation of the dermis and an absence of epidermis (Figure 8). No evidence of bacterial or fungal infection was visible in these sections (H & E). Coincident with this condition were marked posterior kidney changes, likely resulting from osmotic stress invoked by the lesions. Several fish featured mild glomerular changes with vacuolation and proteinaceous/hyaline casts deposited in the glomeruli (Figure 9). The proximal tubule also contained proteinaceous material in some fish. More severe accumulations of hyaline material in the glomeruli were accompanied by glomerular swelling, dilation of the Bowman's space, and accumulations of hyaline material in the epithelium of both the proximal and distal tubules. Tubular and glomerular necrosis was evident in only a single fish, and distinct hyaline deposits were also evident in this fish, associated with the ellipsoids of the spleen and accumulations of melanomacrophages.

Discussion

Lake whitefish collected from Jackfish Bay showed dramatic increases in age to maturity, decreases in gonad size and plasma steroid levels and increased levels of MFO activity. Similar changes have been reported in white sucker collected from this site during 1988-1990 (McMaster et al. 1991a,b; Munkittrick et al. 1991a). Longnose sucker (Catostomus catostomus) also show depressed steroids in females, elevated MFOs in both sexes

(Munkittrick et al. 1991a,b) and delayed maturity (Munkittrick, unpubl. data). These results show that all three benthic species which have been examined in detail (lake whitefish, white sucker, longnose sucker) respond in a similar manner, although the magnitude of changes differ between species.

Prior to secondary treatment, Jackfish Bay lake whitefish exhibited increased liver size relative to two reference sites. During the first year of secondary treatment (1990), liver weights decreased by 37% in females and >50% in males to values relative to those found at the reference sites, although hepatic MFOs were induced. Similar changes were evident in white sucker, where liver size declined 47% in females and 22% in males in 1990 relative to 1989, although the livers were still larger than at the reference sites (Munkittrick, unpubl. data). This indicates that increases in liver size are not required for induction of MFO activity. White sucker collected from 1988-90 exhibited induced MFO activity, as measured by benzo(a)pyrene hydroxylase, diphenyloxazole hydroxylase or EROD (McMaster et al. 1991a; Munkittrick et al. 1991a,b). There was no evidence of improvement in MFO activity following secondary treatment (Munkittrick et al. 1991b). However, there was no evidence of MFO induction in longnose sucker, reduced MFO activity in white sucker and a reduced impact zone for MFO induction in lake whitefish collected two weeks after a planned mill maintenance shutdown (Munkittrick et al. 1991b). These data suggest that a) secondary treatment has not been successful in removing "MFO-active" compounds from BKME, b) induction is not related to sediment contamination with persistent compounds, and that c) the inducing agent(s) are rapidly cleared by fish (Munkittrick et al. 1991b).

Lake whitefish collected from Jackfish Bay in 1989 had reduced gonad size relative to two reference sites. These impacts were consistent in 1990, and correspond well with the reproductive impacts previously identified in Jackfish Bay (Munkittrick et al. 1991a) and at a site receiving BKME in the Baltic Sea (Andersson et al. 1988). The lack of any improvement in the reduced reproductive commitment of lake whitefish from 1989 to 1990 in Jackfish Bay, the reduced levels of circulating sex steroids found in 1990, and the

failure of white sucker gonad sizes or steroid levels to recover during 1990 or 1991 (Munkittrick and Van Der Kraak, unpubl. data) suggests that secondary treatment has had no effect on chronic reproductive impacts.

Examination of the ovaries from lake whitefish in August 1990, indicate that females from Jackfish Bay have significantly higher fecundity relative to the reference site. Although these fish have more eggs, their gonadal weights are significantly lower, indicating that eggs from Jackfish Bay are considerably smaller than those in reference females. White sucker from Jackfish Bay also have reduced egg size (McMaster et al. 1991b) but have reduced fecundity with age (Munkittrick et al. 1991a). Although white sucker eggs were smaller at the BKME site, this had no effect on larval survival following hatch (McMaster et al. 1991b). However, white sucker migrate to clean water to spawn, allowing the most sensitive stages of larval development to occur in uncontaminated waters. Whitefish however, spawn in the lake and larval development may occur in contaminated areas. Whether there are impacts on early developmental life stages of lake whitefish is unknown.

Although MFO induction, gonad size and steroid levels have not recovered during the first year of secondary treatment, the installation of an aerated lagoon (October 1989) and process changes since 1988, have reduced daily effluent discharges at the 1200 tonne per day mill from >35000 kg BOD, >5800 kg total suspended solids (TSS), 59.4 kg phosphorus (Ontario Ministry of Environment 1988) and 2773 kg AOX (Environment Canada 1991) to 1500 kg BOD, 4145 kg TSS, 47.3 kg phosphorus and 1970 kg AOX (Environment Ontario 1991). These reductions correspond to removal of 95% of BOD and 20-30% of the TSS, phosphorus and AOX. This has resulted in elimination of acute lethality of the effluent to fish caged in Jackfish Bay (K. Flood, Ontario Ministry of Environment, Toronto, ON M4V 1P5; unpubl. data) and has improved water clarity in Jackfish Bay and reduced the temperature of the discharge substantially. However, the extent to which these changes effect the number of chronic impacts identified at Jackfish Bay, is unknown.

More than 20% of the lake whitefish showed external lesions, in an equal frequency on both sides and sexes. The lesions were predominantly unilateral and never symmetrical, and most penetrated the body cavity without damage to underlying organs. The relative absence of inflammation suggests no infectious etiology for these lesions, and/or a reduced ability to mount an immune response. The lesions are not seen on round whitefish (Prosopium cylindraceum), lake trout, lake herring (Coregonus artedii), white sucker, longnose sucker or burbot (Lota lota), which are commonly captured in Jackfish Bay. Small numbers of pike (Esox lucius), chinook salmon and walleye (Stizostedion vitreum) have also been collected at Jackfish Bay, but do not show external lesions.

Similar wounding has been reported on lake whitefish for predatory attack by cormorants, loons and other fish-eating birds, but wounds associated with a successful escape from a predator usually run from anterior to posterior, not from dorsal to ventral. There is no known commercial fishing activity in Jackfish Bay and the wounds are too far posterior to be associated with gill net marks. The presence of apparently fresh wounds in the population at the same time as healed scars, suggests that some fish survive the wounding and that wounding is taking place at more than one time of year; wounds have been seen in Jackfish Bay during May, August and September sampling periods, and during 1989, 1990 and 1991 sampling years (Munkittrick, unpubl. data). The wounds would not appear to be mechanical since they are always present on the body cavity, follow the same angle, and are not present on any other species captured in Jackfish Bay. Since the lake whitefish were collected from relatively deep water in August, prior to spawning, there is no apparent explanation for the lesions.

The presence of these lesions in an isolated, unpopulated bay which has received large volumes of pulp mill effluent for more than 40 y, as well as the absence of reports of similar wounding in other lake whitefish (Reist et al. 1987) suggests an association with the discharge of BKME. Photographs were distributed to numerous fisheries groups, including the Ontario Ministry of Natural Resources (Thunder Bay), Ontario Ministry of Environment (Thunder Bay), Canadian Wildlife Service (Burlington), Department of

Fisheries and Oceans (Winnipeg) and commercial fishermen on Lake Superior. Commercial fishermen collecting more than 10,000 whitefish per year from other areas of Lake Superior did not recognize the lesions. In May, 1991, lake whitefish with similar lesions were collected from around the mouth of the Kaministikwia (Kam) River, Thunder Bay (Lake Superior) by personnel of the Lake Superior Fisheries Assessment Unit (Pat Furlong, Ontario Ministry of Natural Resources, Thunder Bay, pers. comm.); the Kam River receives more than 200,000 $m^3 d^{-1}$ of primary-treated BKME. Although commercial fishermen estimated the frequency of wounding at this site to be 20%, fish collections by the assessment unit found 18/224 whitefish to be lesioned. Several specimens (7) were sent to our laboratory, and the lesions appear similar to those seen in Jackfish Bay (Figure 10).

Summary

Lake whitefish exposed to BKME exhibited reduced gonad sizes, delayed age to maturation, decreased levels of the plasma sex steroids testosterone and 17β-estradiol, induced hepatic mixed function oxygenase (MFO) activity. Liver size was smaller following operation of the secondary treatment system, but there was no other evidence of recovery during the first year of secondary treatment. The disappearance of MFO activity during a shutdown (Munkittrick et al. 1991b) suggests that historical sediment contamination is not responsible for the observed physiological changes. More than 20% of the whitefish collected at the BKME site exhibited lateral, slash-like lesions which penetrated the body cavity. Histological examination revealed no evidence of an infectious etiology, and the wounds could not be accounted for by known causes. Similar lesions were found in 1991 near a second BKME discharge on Lake Superior. The restriction of these lesions to two sites receiving BKME, and the correlation with other adverse impacts suggests that the BKME discharge may be the causative agent.

Acknowledgements

MFO measurements were conducted by Lynn Luxon (DFO, Burlington). Funding for this study was provided by both the Department of Fisheries and Oceans and the Ontario Ministry of Environment Research Advisory Council. The assistance of Dave Rokosh (Water Resources Branch, OME, Rexdale, Ontario) was valuable. Field collections were supervised by Leo and Maye Marchand (Marchand Fisheries, Dorion, Ontario), and the assistance of Linda Melnyk-Ferguson (Ontario Ministry of Natural Resources, Terrace Bay, Ontario) and Lorne Townes (Ontario Ministry of Natural Resources, Nipigon, Ontario) are gratefully acknowledged. The samples and information provided by Pat Furlong, Lake Superior Fisheries Assessment Unit, Ontario Ministry of Natural Resources (Thunder Bay, Ontario), were appreciated. This report was prepared for the Ontario Ministry of Environment (MOE), and the Department of Fisheries and Oceans (DFO), as part of MOE and DFO-funded projects related to Jackfish Bay. The views and ideas expressed in this report are those of the authors and do not necessarily reflect the views and policies of the provincial or federal government, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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Table 1. Scale of lesion severity. The numerical scale does not represent stages of development of the lesions, but was meant to characterize lesions for assessment of frequency.

Stage	Description
1	possible new lesion, not open
2	new lesion, not through to peritoneal cavity
3	lesion into peritoneal cavity, edges sharply defined
4	edges of wound not sharp
5	some healing has occurred
6	new scar covers lesion
7	old scar, well healed

Table 2. Age, length, liver, gonad and body weights for lake whitefish collected in August, 1989 and August and September, 1990 from the BKME-exposed (Jackfish) and reference (Black and Mountain) sites. Values are expressed as mean ± SE (n), asterisks denote a significant difference of the reference site from Jackfish Bay (BKME) for that year as analyzed by ANOVA using log transformed values (* $p<0.05$; ** $p<0.01$; *** $p < 0.001$).

Site	Date	Age (y)	Length (cm)	Weight (g)	Liver (g)	LSI[1]	Gonad (g)	GSI[2]
Males								
Black	1989		46.3 ± 0.9 (15)	1445 ± 87 (15)	10.7 ± 1.0 (15)***	0.73 ± 0.04 (15)	28.6 ± 1.9 (15)***	2.02 ± 0.07 (15)
Mountain	1989		40.3 ± 1.4 (7)*	1039 ± 111 (7)*	11.0 ± 1.5 (7)***	1.07 ± 0.10 (7)	17.9 ± 2.6 (7)***	1.74 ± 0.13 (7)
	1990	8.95 ± 0.4 (14)**	42.7 ± 0.9 (18)	996 ± 63 (18)	9.0 ± 0.6 (18)*	0.93 ± 0.05 (18)	15.2 ± 1.9 (18)***	1.47 ± 0.11 (18)
Jackfish	1989		45.4 ± 1.5 (14)	1491 ± 143 (14)	29.2 ± 3.5 (14)	1.94 ± 0.14 (14)	7.3 ± 1.4 (14)	0.46 ± 0.06 (14)
	1990	7.6 ± 0.3 (32)	42.1 ± 0.7 (31)	999 ± 53 (31)	13.7 ± 1.4 (25)	1.35 ± 0.10 (25)	5.6 ± 1.4 (24)	0.46 ± 0.09 (24)
	September		43.7 ± 1.9 (9)	1211 ± 100 (10)	16.1 ± 1.7 (6)	1.53 ± 0.14 (6)	15.2 ± 4.3 (9)	1.19 ± 0.34 (9)
Females								
Black	1989		46.2 ± 0.7 (16)	1517 ± 60 (16)	16.3 ± 1.0 (15)***	1.16 ± 0.06 (15)	73.1 ± 8.0 (15)**	5.06 ± 0.49 (15)
Mountain	1989		42.1 ± 1.0 (16)**	1243 ± 67 (16)**	19.8 ± 1.8 (16)***	1.62 ± 0.07 (16)	51.6 ± 5.7 (16)**	4.24 ± 0.29 (16)
	1990	8.9 ± 0.5 (16)	41.6 ± 0.9 (17)	966 ± 82 (17)	15.5 ± 3.4 (17)	1.80 ± 0.49 (17)	47.3 ± 5.4 (16)**	5.00 ± 0.36 (16)
Jackfish	1989		45.9 ± 1.1 (16)	1520 ± 84 (16)	36.4 ± 4.5 (16)	2.41 ± 0.21 (16)	39.4 ± 5.7 (16)	2.58 ± 0.31 (16)
	1990	8.31 ± 0.4 (26)	42.9 ± 0.7 (27)	1125 ± 71 (27)	16.9 ± 1.9 (24)	1.52 ± 0.13 (24)	30.5 ± 5.8 (24)	2.45 ± 0.33 (24)
	September		42.1 ± 1.7 (10)	1124 ± 133 (10)	21.4 ± 1.9 (8)	2.22 ± 0.20 (8)	46.6 ± 23.3 (10)	3.78 ± 1.81 (10)

[1] LSI= liversomatic index = 100 * liver weight/(body weight-liver weight)

[2] GSI= gonadosomatic index = 100 * gonad weight/(body weight-gonad weight)

Legend to Figures

Figure 1. Jackfish Bay. The effluent from the bleached kraft mill enters Blackbird Creek, and flows 15 km to Moberley Bay, the western arm of Jackfish Bay. Most of the whitefish were collected near Little Nick rock, approximately 1.8 km off the mouth of Blackbird Creek.

Figure 2. Liver weight versus body weight for male (a) and female (b) whitefish collected from the BKME site in 1989 (boxes) or 1990 (crosses) relative to two reference sites (Mountain, stars; Black Bay, asterisks).

Figure 3. Gonad weight versus body weight for male (a) and female (b) whitefish collected from the BKME site (crosses) and reference sites (Mountain, stars; Black Bay, asterisks). The figure includes pooled data from 1989 and 1990 sampling.

Figure 4. Levels of plasma testosterone in males and testosterone and 17β-estradiol (ng mL^{-1}) in female lake whitefish from Jackfish Bay (BKME; shaded) and Mountain Bay (Ref; open) during August, 1990.

Figure 5. Hepatic EROD activity (pmol resorufin min^{-1} mg $protein^{-1}$) of male and female whitefish collected from Jackfish Bay (shaded) and Mountain Bay (open) during August, 1990.

Figure 6. Lesioned lake whitefish collected from Jackfish Bay in August, 1990. The lesions were always on the body cavity and were present on more than 20% of whitefish examined.

Figure 7. Distribution and severity of lesions recorded in lake whitefish during August, 1990. External lesions were subjectively categorized on a scale of 1 to 7, with

increasing numbers from 1 to 5 reflecting a progressive increase in the size and severity of the lesion, and with numbers greater than 5 reflecting progressive healing.

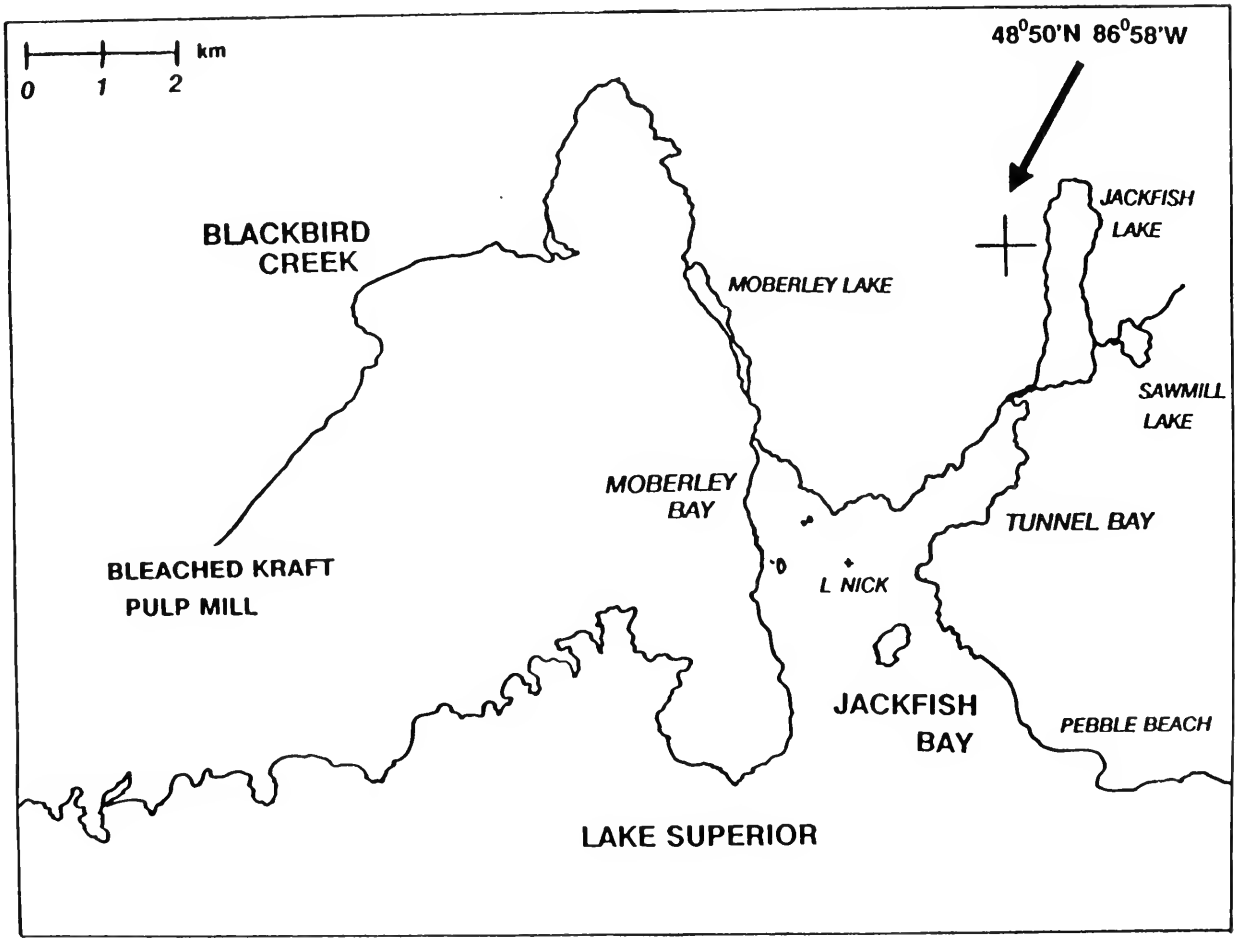
Figure 8. Histological section through lateral "slash" showing intact muscle bundles among collagenous connective tissue. Note absence of inflammatory response. H&E, 1000 x mag.

Figure 9 a) normal renal glomeruli showing mild post-mortem (autolytic) vacuolation. Note size of glomerulus relative to adjacent renal tubules and size of normal Bowman's space. H&E, 2000 x mag.

b) renal glomeruli from whitefish with lateral "slash", showing distension of Bowman's space, glomerular swelling and distension due to deposits of hyaline material adjacent to capillary endothelium. Note enlargement of glomeruli relative to adjacent tubule. H&E, 2000 x mag.

c) renal glomeruli from whitefish with lateral "slash", showing distension of Bowman's space, glomerular swelling and distension due to deposits of hyaline material adjacent to capillary endothelium. Note enlargement of glomeruli relative to adjacent tubule. H&E, 1000 x mag.

Figure 10. Lake whitefish collected from the mouth of the Kam River, Thunder Bay, Lake Superior, showing the same type of wounding as seen in Jackfish Bay. The Kam River receives more than 200,000 m^3 d^{-1} of BKME.


km
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2
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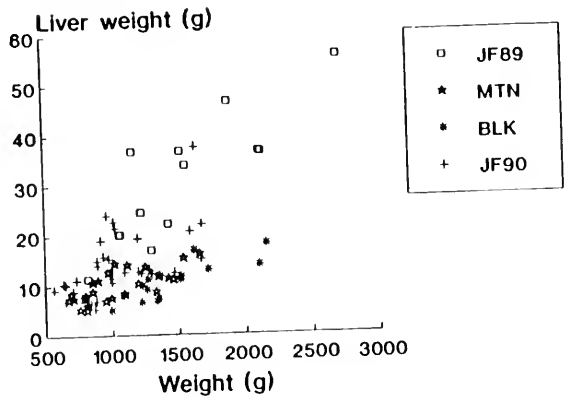
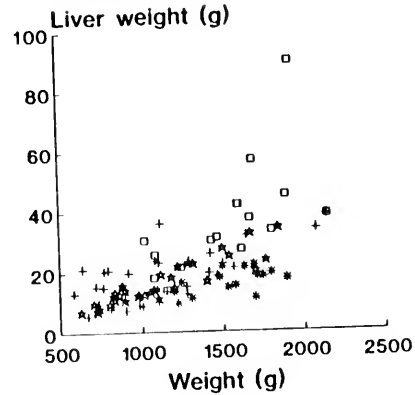
JACKFISH LAKE
BLACKBIRD CREEK
MOBERLEY LAKE
SAWMILL LAKE
MOBERLEY BAY
TUNNEL BAY
BLEACHED KRAFT PULP MILL
L NICK
JACKFISH BAY
PEBBLE BEACH
LAKE SUPERIOR

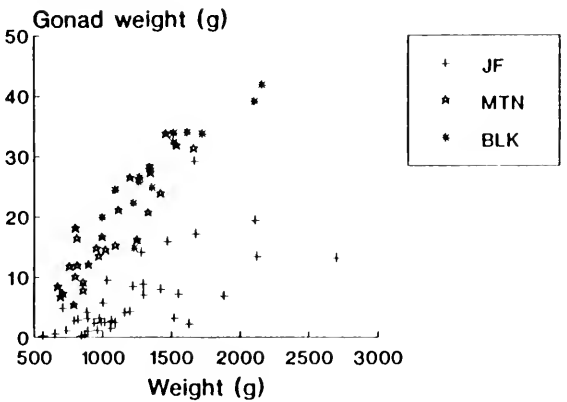
a) Males



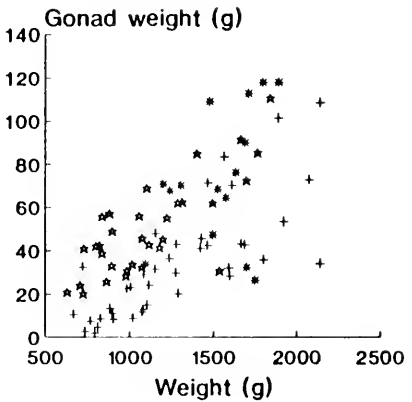
b) Females

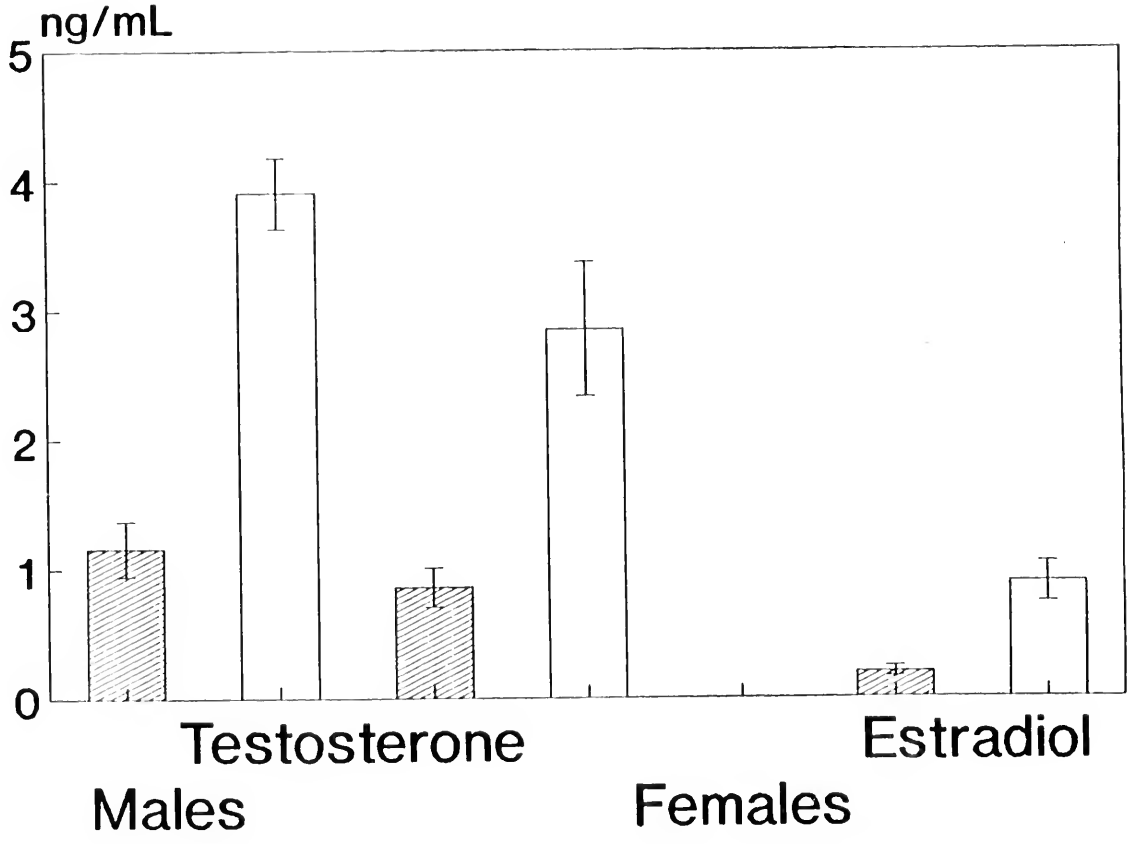


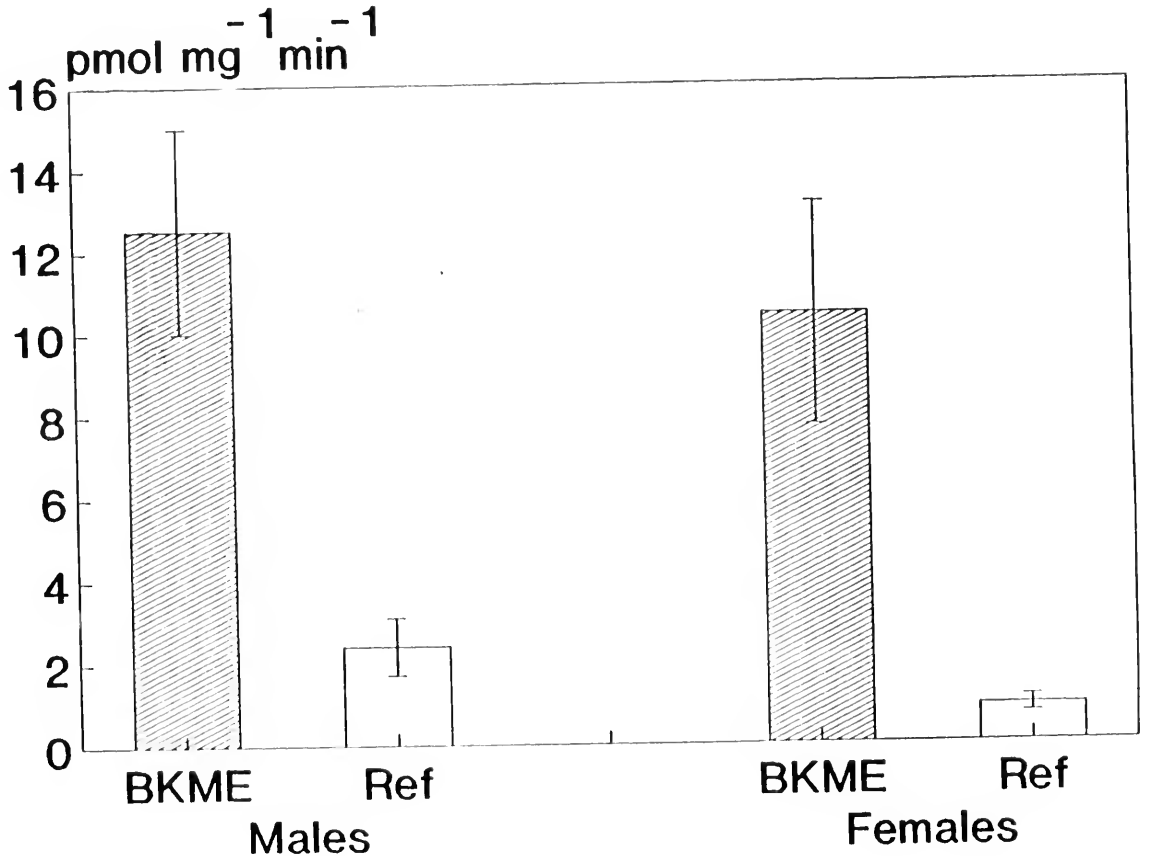
a) Males



b) Females




ng/mL
5
4
3
2
1
0
Testosterone
Estradiol
Males
Females


pmol mg -1 min -1
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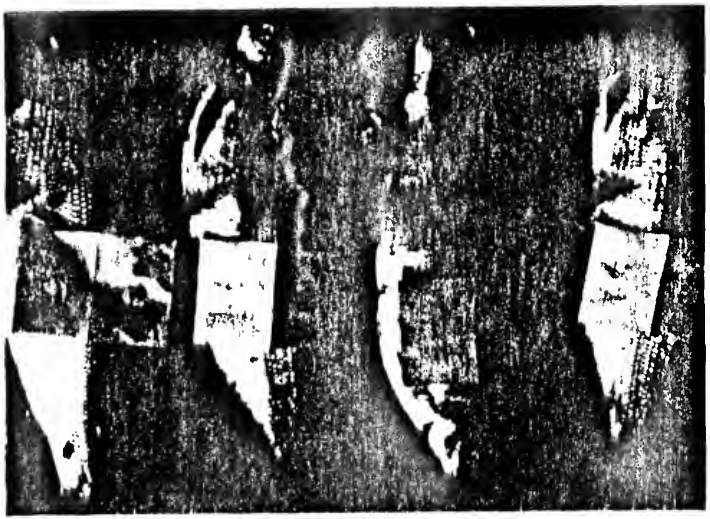
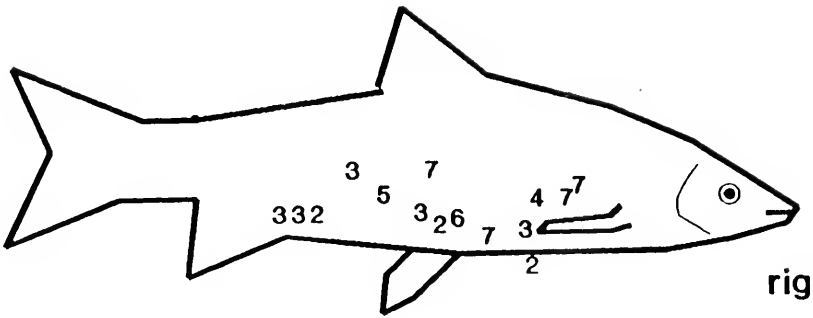
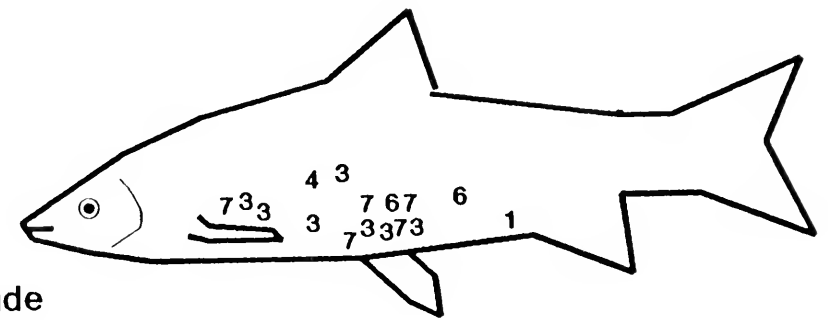




right side



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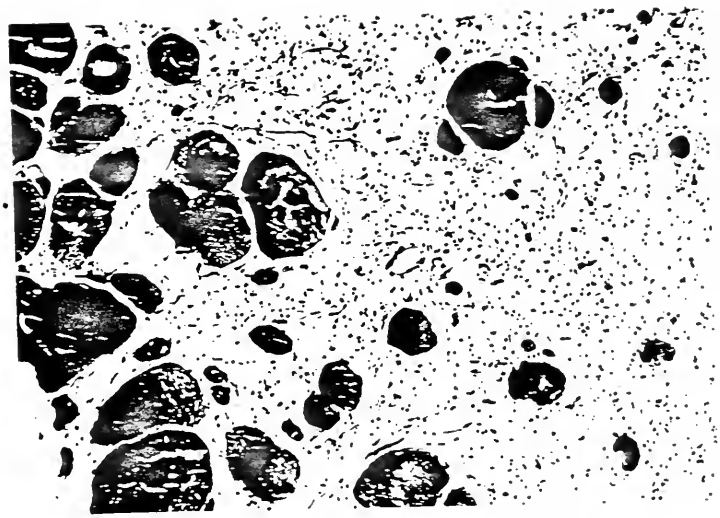

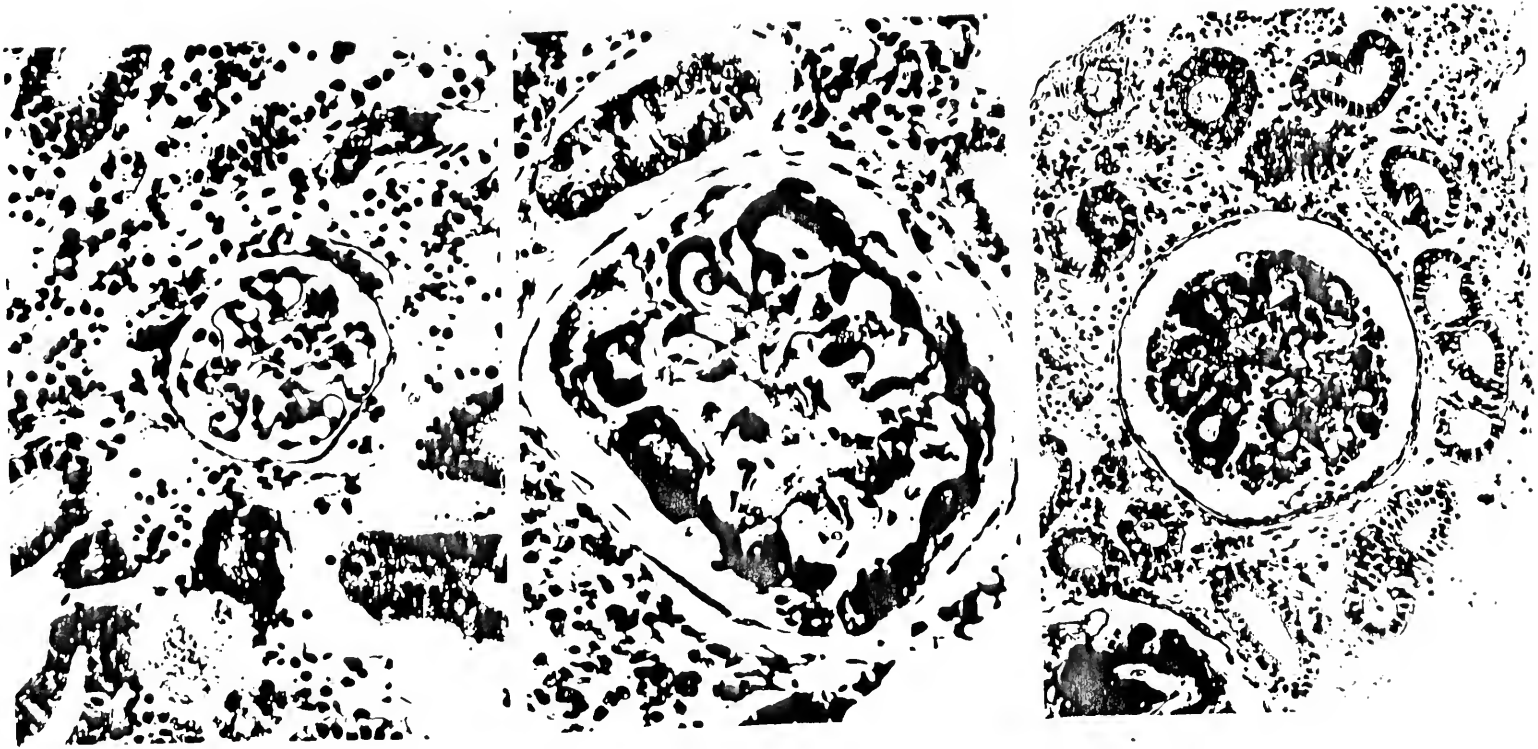

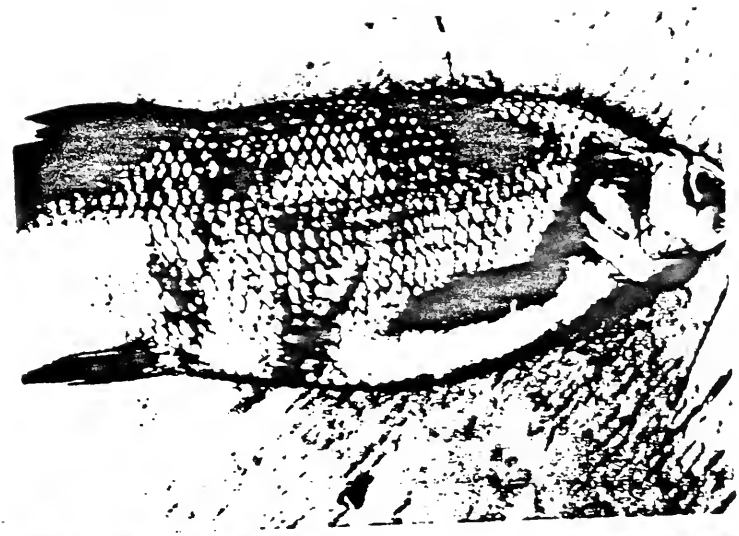

Reproductive dysfunction and MFO activity in three species of fish exposed to bleached kraft mill effluent.

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Abstract

Our recent studies have demonstrated reproductive dysfunction in white sucker (Catostomus commersoni), longnose sucker (C. catostomus) and lake whitefish (Coregonus clupeaformis) populations exposed to bleached kraft mill effluent (BKME). All three species show elevated levels of hepatic mixed function oxygenase (MFO) activity and depressed circulating steroid levels. White sucker and lake whitefish exhibit delayed sexual maturity, changes in fecundity and reduced secondary sexual characteristics. MFO activity in white sucker, as measured by three catalytic assays, has not changed with installation of secondary treatment. However, samples collected after a two week maintenance shutdown showed return of MFO activity to reference levels in both longnose sucker and lake whitefish, whereas white sucker showed a 50% decline. The relationship between elevated MFO activity and depressed steroid levels is unclear, but preliminary results suggest that the two phenomena are not directly linked. White sucker show depressed steroid production and impaired regulation, independent of MFO activity.

Keywords: bleached kraft mill effluent; MFO activity; EROD; testosterone; estradiol

Introduction

During the latter part of the 1980's, attention was focused on effluents from bleached kraft mills (BKME) by two major developments: a) the identification of dioxins and furans in receiving waters downstream of bleached kraft pulp mill discharges (Voss et al., 1988; NCASI, 1990), and b) the identification of low-level impacts of BKME on fish community structure, growth, carbohydrate metabolism, maturation, recruitment and mortality of fish near a Scandinavian BKME discharge (reviewed in Sodergren, 1989). The Scandinavian studies have been criticized in part because of the absence of supporting data from North American studies, the presence of confounding industrial discharges and habitat conditions in the Baltic region, and the operational condition of the mill during the study period (Sprague and Colodey 1989).

Detailed North American studies have been slow to develop. The Ontario Ministry of Environment (OME) initiated a survey of the activity of hepatic mixed function oxygenase (MFO) enzymes near steel mills and bleached kraft pulp mills during 1987 (Smith et al., 1991). Elevated MFO activity has been consistently reported with BKME exposure in the Scandinavian studies (Andersson et al., 1988; Lindstrom-Seppa and Oikari, 1990; Oikari et al., 1985). The OME study was expanded in 1988 to examine other indications of fish population health near a BKME discharge (Munkittrick et al., 1991a). These fish health studies have concentrated on Jackfish Bay, a relatively isolated bay on the north shore of Lake Superior. Jackfish Bay has been identified as an area of concern by the International Joint Commission, due to the effects of the effluent from a 1200 metric tonne per day bleached kraft mill at Terrace Bay (IJC, 1989). From 1949 until the 1970's, the effluent received little or no treatment. The mill underwent a full bleaching expansion in 1972, and incorporated primary effluent treatment during a 1978 mill expansion (Farara et al., 1988). Secondary

treatment installation was completed during September, 1989; current discharges approximate 120,000 m^3 d^{-1}. The effluent now passes through two primary clarifiers before entering an aerated lagoon which has 36 aerators, a volume of approximately 1.2 x 10^9 L and an 8-10 d retention time.

Studies conducted during 1988 (Munkittrick et al. 1991a) and 1989 (McMaster et al. 1990a,b) were completed when the mill effluent received only primary treatment. Present studies have been characterizing changes in the fish populations after the mill initiated secondary treatment of the effluent in September 1989.

Review

Collections conducted during primary treatment of the BKME (McMaster et al. 1991a,b; Munkittrick et al. 1991a) found that white sucker (Catostomus commersoni) showed delayed maturity, smaller gonads, reduced egg size, increased condition factor, increased liver size, fewer secondary sexual characteristics and a smaller size at age than white sucker from comparable reference sites. Furthermore, there were increased levels of hepatic mixed function oxidase (MFO) activity (as measured by catalytic activity against benzo(a)pyrene, diphenyloxazole and ethoxyresorufin) and reduced levels of circulating steroids (as measured by testosterone, 11-ketotestosterone, 17β-estradiol and 17α20β-dihydroprogesterone), relative to fish at reference sites.

Similar changes have been documented in longnose sucker (Catostomus catostomus) and lake whitefish (Coregonus clupeaformis) collected from Jackfish Bay (Munkittrick et al. 1991b,c). Collections on the St. Maurice River, downstream from a primary-treated bleached kraft mill at La Tuque, Quebec have also reported increased MFO activity and decreased testosterone levels in male white sucker (M. Gagnon and G.J. Van Der Kraak, unpubl. data; Hodson et al. 1991). Impacts

of BKME on MFO activity have also been reported from other bleached kraft mills in Ontario (Munkittrick et al. 1991b; Servos et al. 1991; Smith et al. 1991), Alberta (Swanson, pers. comm.) and B.C. (Rogers et al. 1989).

At Jackfish Bay, we have found increased levels of hepatic MFO activity during August in white sucker (24-fold), longnose sucker (18-fold), lake whitefish (8-fold) and lake trout (7-fold) collected from Jackfish Bay (Munkittrick et al. 1991b). MFO activity levels, measured as EROD, PPO or B(a)P have not declined in white sucker since the initiation of secondary treatment (Munkittrick et al. 1991b). Despite the absence of a change with secondary treatment, EROD activity levels in longnose sucker and lake whitefish declined from 18-fold induction and 7-fold induction to reference levels within two weeks of a planned maintenance shutdown (Munkittrick et al. 1991b). Declines in white sucker were approximately 50% in activity levels during shutdown, and during a caging study where white sucker were collected from the effluent and transferred to clean water for 4 d (Munkittrick et al., 1991b).

Despite the similarity in biochemical response of the three species (increased MFOs, decreased steroids), there are quite dramatic differences in changes at the level of the whole organism. Lake whitefish show as much as a 4-5 y delay in maturity of males, and a 4-fold decrease in egg size in females. Fewer than 10% of whitefish collected are sexually mature, despite the absence of a difference in fish size between sites (Munkittrick et al. 1991c). White sucker show a 2 to 4 year delay in maturity, and approximately a 25% decrease in gonadal size (McMaster et al. 1991a; Munkittrick et al. 1991a). Longnose sucker show no changes in gonadal size (Munkittrick et al. 1991b). All three species show approximately a three-fold depression of steroid levels in female fish. Both white sucker and lake whitefish males show similar 3-fold depressions in circulating levels. Longnose sucker males do not show lower steroid levels, although these samples were

collected during mill shutdown, when biochemical responses are different (Munkittrick et al. 1991b).

The consequences of the changes in gonad size and delayed maturity are unknown. Male whitefish mature at the BKME site between the ages of 10 and 11, relative to 6 at the reference sites (Munkittrick et al., 1991c). Similarly, the combination of decreased gonadal size, delayed maturity and decreased fecundity with age may combine to greatly reduce the reproductive output of white sucker (McMaster et al., 1991a; Munkittrick et al., 1991a).

Only the white sucker have been evaluated for reproductive performance thus far, and there was no evidence of a depression of fertilization (McMaster et al., 1991b). Although spermatozoan motility was reduced, there were no differences in seminal plasma constituents, sperm volume or concentration, and males performed equally well in fertilization tests. Larvae hatched from eggs collected at the contaminated site exhibited no difference in mean survival or developmental times, but grew at a slower rate compared to reference larvae (McMaster et al., 1991b). No difference in larval MFO activity was detected between sites at 21 d posthatch.

All changes which are evident in adult fish (decreased secondary sexual characteristics, delayed maturation, reduced gonad size) can be correlated with reduced steroid levels, but it is unclear if the altered steroid levels can be directly related to the induced levels of MFO activity. If the induced MFOs were responsible for the reduction in circulating steroids, one would expect fish with higher activity to show lower steroid levels. There is no relationship between level of MFO activity and steroid levels, within site (McMaster, 1991). Furthermore, steroid changes are still evident during spawning, when MFO levels are low (the white sucker ascend uncontaminated streams to spawn) (McMaster et al., 1991a; Munkittrick et al., 1991a) and during mill operational shutdown, when MFO activity is reduced to reference levels

(Munkittrick et al., 1991b).

Recent studies have demonstrated impacts of BKME at multiple sites in the regulation of steroid production and metabolism (hypothalamic-pituitary-gonadal axis). Prespawning white sucker from the BKME site were unresponsive to administration of an exogenous gonadotropin releasing hormone (D-Arg^{6}, Pro^{9}N-Et sGnRH) (Van Der Kraak et al., 1991). As well, fish from the BKME site did not exhibit an increase in testosterone or 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-P) secretion in response to the GnRH analog (Van Der Kraak et al., 1991). In vitro incubations of ovarian follicles obtained from fish at the BKME site revealed depressed basal secretion of testosterone and 17α20β-P and diminished responsiveness to human chorionic gonadotropin and forskolin relative to ovarian follicles from the reference site (Van Der Kraak et al., 1991). Depressed levels of testosterone and 17α,20β-P glucuronides in BKME exposed fish also suggest that there are effects on the peripheral metabolism of steroids (Van Der Kraak et al., 1991). These changes occur despite the fact that these fish have left the BKME-exposed area, and are spawning in clean water.

Summary

Three years of studies have shown that

a) MFO levels are induced and steroid levels reduced in all three species of fish examined,

b) in lake whitefish and white sucker, the reduced steroid levels correlate with delayed maturity, and reduced gonad size, secondary sex characteristics and fecundity,

c) steroid changes do not appear to be directly linked to induced MFO levels or increased catalytic rate, but are related to decreased production and altered regulation,

d) in longnose sucker, steroid abnormalities persist further from the effluent, and occur during mill shutdown when MFO levels are not induced, and

e) secondary treatment does not appear to remove the compounds responsible for MFO induction, but it is too soon to evalue the reproductive conseuqences of improved effluent treatment.

Acknowledgements

Funding for these studies was provided by both the Department of Fisheries and Oceans and the Ontario Ministry of Environment Research Advisory Council (Grants 463G and 494G). The assistance of Ian Smith and Dave Rokosh (Water Resources Branch, OME, Rexdale, Ontario) is gratefully acknowledged. The views and ideas expressed in this manuscript are those of the authors and do not necessarily reflect the views and policies of the provincial or federal government.

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Longterm studies of bleached kraft mill effluent (BKME) impact on fish: response of hepatic mixed function oxygenase (MFO) activity and plasma sex steroids to secondary treatment and mill shutdown.

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ABSTRACT

The discharge of bleached kraft mill effluent (BKME) into Jackfish Bay, Lake Superior, has been associated with a number of changes in the physiology and whole organism responses of four fish species. Current studies have been following physiological indicators of BKME impact for evidence of improvement after the installation of a $20M secondary treatment system, which began operation during October, 1989. White sucker (Catostomus commersoni) collected from Jackfish Bay during August 1990 exhibited similar hepatic mixed function oxygenase (MFO) activity as recorded in samples collected during August of 1988 and 1989. Secondary treatment has not been successful in eliminating BKME impacts on MFO activity. Hepatic MFO activity was also induced in both longnose sucker (Catostomus catostomus) and lake whitefish (Coregonus clupeaformis) in August 1990. However, samples collected two weeks after a planned mill maintenance shutdown during September 1990, showed no MFO induction in longnose sucker, reduced MFO activity in white sucker and a reduced impact zone for MFO induction in lake whitefish. A reduction in circulating levels of gonadal sex steroids has also been recorded in fish exposed to BKME in Jackfish Bay. Neither secondary treatment nor mill shutdown were successful in eliminating impacts of BKME exposure on levels of testosterone and 17β-estradiol in female white sucker and longnose sucker. The short duration of MFO induction after shutdown and the persistence of steroid reductions suggest that a) secondary treatment has not been successful in removing "MFO-active" compounds from BKME, b) induction is not related to sediment contamination with persistent compounds, c) the inducing agent(s) are rapidly cleared by fish and that d) effects on steroids may not be directly related to MFO induction.

Keywords: bleached kraft pulp mill effluent, secondary treatment, MFO activity, EROD, testosterone, 17β-estradiol

INTRODUCTION

Hepatic mixed-function oxygenase (MFO) enzyme activity has been proposed as an indicator for delineating zones of exposure to inducing agents (1,2). Induced MFO activities have been found in fish downstream of bleached kraft mills in Ontario [3-5], other Canadian provinces [6,7] and Scandinavia [8-10]. The chemicals responsible for the enzyme induction have not been identified, although polychlorinated dibenzofurans and dioxins (PCDF/Ds) have been implicated as a causative agent for the induced activity near bleached kraft pulp mills [6,7].

Over the past three years, we have been studying the impacts of bleached kraft mill effluent (BKME) on Jackfish Bay, Lake Superior; Jackfish Bay received untreated or primary-treated BKME from 1949 until 1989. The study site is isolated and receives no other industrial or domestic effluents. Studies conducted in 1988 [4] and 1989 [11,12] examined fish responses when the BKME received only primary treatment to remove fibre and suspended solids. Identified impacts of primary-treated BKME were induced MFO activity, as well as depressed plasma levels of gonadal sex steroids (testosterone, 11-ketotestosterone, 17α20β-dihydroxy-4-pregnen-3-one and 17β-estradiol), delayed age to maturation, altered fecundity and reduced secondary sexual characteristics in both white sucker (Catostomus commersoni) [4,11,12] and lake whitefish (Coregonus clupeaformis) [13]. Altered sex steroid levels have also been observed in white sucker collected downstream of a bleached kraft mill in Quebec [6; M. Gagnon and G. Van Der Kraak, unpubl. data]; however the influence of altered levels of steroids on reproductive success is not understood.

Proposed legislation under the Canadian Fisheries Act and the Canadian Environmental Protection Act will require all pulp mills to produce effluent which is not acutely lethal to fish [2]. In order to achieve this, most mills will likely have to install some type of secondary treatment system, at an approximate cost of $20-30 M. There has been no confirmation that secondary treatment will remove the compounds responsible for the sub-lethal biological effects identified in Jackfish Bay. The present studies have continued observations at Jackfish Bay after the mill installed a secondary treatment aerated lagoon, which began operation in October 1989. The installation of an aerated lagoon and process changes since 1988 have reduced daily effluent discharges at the 1200 air dried metric tonnes (ADMT) per day mill from >35000 kg BOD, >5800

kg total suspended solids (TSS), 59.4 kg phosphorus [14] and 2773 kg AOX [15] to 1500 kg BOD, 4145 kg TSS, 47.3 kg phosphorus and 1970 kg AOX [16]. These reductions correspond to removal of 95% of BOD and 20 to 30% of the TSS, phosphorus and AOX. This has resulted in elimination of acute lethality of the effluent to fish caged in Jackfish Bay (K. Flood, Ontario Ministry of Environment, Toronto, ON, Canada M4V 1P5; unpubl. data) and has improved water clarity in Jackfish Bay and reduced the temperature of the discharge substantially.

This paper compares MFO activity in wild fish collected prior to (August 1988 and 1989) and after the initiation of secondary treatment of effluent (August 1990). In addition, the persistence of hepatic MFO activity and plasma steroids were evaluated during a scheduled mill maintenance shutdown in September 1990.

MATERIALS AND METHODS

Locations and Collections

Jackfish Bay has been identified as an area of concern by the International Joint Commission, due to the effects of the effluent from a 1200 ADMT per day bleached kraft mill at Terrace Bay [17]. The 120,000 $m^3 d^{-1}$ of BKME composes 65 to 95% of the volume of Blackbird Creek, which carries the effluent 15 km to Moberley Bay, the western arm of Jackfish Bay. From August 12 to 15 and September 27 to 30 1990, fish were collected in gill nets at sites 0.7 km (plume; mean depth 6 m), 1.8 km (Little Nick rock; mean depth 25 m), 3.3 km (St. Patrick Island; mean depth 18 m) and 5.2 km (Pebble Beach in September only; mean depth 20 m) to the southeast of the mouth of Blackbird Creek (Figure 1). Reference collections were conducted in Mountain Bay (August 9 to 11; latitude 48°56'N longitude 87°50'W) or Black Bay (October 1 to 3; 48°30'N, 88°40'W). Both sites have been used in previous studies, receive no industrial discharges and have low levels of MFO activity; they are fully described in [12].

White sucker, longnose sucker (Catostomus catostomus), lake whitefish and lake trout (Salvelinus namaycush) were collected in Jackfish Bay, but all species were not found consistently at all sampling stations. The failure to capture white sucker at Little Nick rock in September resulted

in their collection from an additional site, Cody Island, which is located 1.3 km southwest of the mouth of Blackbird Creek (Figure 1). Failure to capture sufficient numbers of live lake trout at the reference site resulted in the collection of additional fish outside of Peninsula Harbour, Lake Superior (latitude 48°44'N; longitude 86°4'W), about 60 km east of Jackfish Bay. This region of Lake Superior receives the discharge of a 450 ADMT bleached kraft pulp mill located in Marathon, Ontario. The Marathon mill discharges its primary-treated effluent through a diffuser set on bottom in 6 m of water. Gillnets were set overnight on August 15 to 16 1990, at several sites adjacent to the diffuser. Although these fish can not be used as an additional reference site, they are included for comparison of activity levels.

A regularly scheduled, complete shutdown occurred at the Terrace Bay mill on September 12-20, 1990 for in-plant maintenance. This shutdown included a cessation of effluent discharge from the lagoon, with flow falling from in excess of 100,000 $m^3 d^{-1}$ to a low of 16-1700 on September 13-14. Lagoon flow and suspended solids approached normal operating levels on September 21 (P.T. Jordan, Ontario Ministry of Environment, Thunder Bay P7C 5G6; pers. comm.). The lagoon has an 8-10 d retention time, and the effluent requires an additional 2 d transit to reach Moberley Bay via Blackbird Creek. This resulted in a reduced exposure of fish to effluent in Jackfish Bay during the last two weeks of September 1990. Fish samples were collected from Jackfish Bay between September 27 and 30, 1990.

Sample analyses

Blood was collected from live fish via caudal puncture into 5.0 mL heparinized vacuutainers and placed on ice for 6 to 8 h. The plasma was collected after centrifugation at maximum speed on an IEC centrifuge for 7 min, frozen in liquid nitrogen and stored at -80°C prior to analysis. Fork length (cm), total weight, liver weight and gonad weight (g) were determined for each fish. Approximately 1 g of liver was placed in cryovials and frozen immediately in liquid nitrogen. The left operculum of each fish was removed, frozen, boiled later to remove the skin, and the annuli counted to determine the age.

In the laboratory, the liver samples were thawed on ice and analyzed for MFO activity using

ethoxyresorufin, diphenyloxazole (PPO) and benzo(a)pyrene (B(a)P) as substrates. Ethoxyresorufin-o-deethylase (EROD) determinations were conducted using a modification of methods described elsewhere [7,18] for activity determinations on post-mitochondrial supernatant (PMS), and detection limit for white sucker hepatic EROD activity was 1.2 pmol resorufin min^{-1} mg^{-1} protein. EROD determinations from 1989 samples were measured on liver samples (PMS) stored at -80°C from August 1989 until October 1990. PPO metabolism was determined using the methodology of Luxon et al. [19] as modified previously [3,4,12], and were expressed as fluorescence units (FU) min^{-1} mg^{-1} protein. B(a)P analyses determined the fluorescence of the metabolic product (3-OH B(a)P) [3,4,12] and results were expressed as either FU min^{-1} mg^{-1} protein in 1988 [4] or pmol 3-OH B(a)P min^{-1} mg^{-1} protein in 1989 [12]. B(a)P analyses in 1990 were conducted using ^{3}H-B(a)P by D. Metner and Dr. L. Lockhart (Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MN, Canada R3T 2N6), and results were expressed as pmol 3-OH B(a)P min^{-1} mg^{-1} protein. The results from 1988 and 1989 analyses for B(a)P and PPO have been previously described, but are presented here for comparison.

Testosterone and 17β-estradiol were measured by radioimmunoassay (RIA) in plasma following ether extraction. A description of the antisera used to measure testosterone and 17β-estradiol were reported in Van Der Kraak et al. [20,21] and the RIA protocols are described in Van Der Kraak et al. [20]. All plasma samples were assayed in duplicate and interassay variability was less than 15% in both RIA systems.

Statistics

Steroid, age and MFO data were compared by the non-parametric Kruskall-Wallis test, while the relationships of length, gonad size and liver size with adjusted body weight (body weight- gonad weight) were analyzed by ANCOVA, using log-transformed values with location of capture as the covariate. Analyses of site differences for length, weight, liver size and gonad size showed significant interactions between both season and sex (white sucker) or site and sex (longnose sucker) ($p<0.01$). Individual comparison within site or season and sex were conducted by Kruskall-Wallis.

RESULTS

Assays for MFO activity during the period of primary treatment (1988 to 1989) were based on the catabolism of B(a)P and PPO (all samples were collected during the month of August, and values are reported only for females). Both assays showed that fish from the BKME site were markedly induced, and showed little variability between years; the units for B(a)P are different between years but the relative induction is similar. EROD analyses were initiated in 1990 due to concern about laboratory safety associated with the B(a)P procedure and the increased sensitivity of the EROD assay. For continuity of data, EROD activity was measured on 1989 samples which had been stored at -80°C, as well as those samples collected during secondary treatment (1990). The EROD activity in samples collected during both years was elevated relative to the Mountain Bay reference site (Figure 2). The initiation of secondary treatment resulted in no change in relative induction levels (exposed/reference) for any of the three catalytic assays (Figure 2).

During August 1990, EROD activity differed markedly between species of fish. Longnose sucker showed the highest EROD activity in the plume and at the reference sites, while lake whitefish showed the lowest EROD activity at both sites (Table 1). White sucker showed the highest induction relative to the Mountain Bay reference site. Lake trout were not collected at either reference site and the Peninsula Harbour data are included for comparison. Lake trout collected from Peninsula Harbour showed mean activity levels of 3 pmol min^{-1} mg^{-1}, while Jackfish Bay lake trout exhibited activities above 21 pmol min^{-1} mg^{-1} (Table 1). Although lake trout were exposed to BKME at both sites, the marked elevation of activity levels at Jackfish Bay suggests induction. Since the longnose sucker collected from Peninsula Harbour are elevated relative to the reference site, we assume that the activity of lake trout samples are not background activity levels.

At Jackfish Bay, there were species differences in the response to distance from the mouth of Blackbird Creek. In August, EROD activity of longnose sucker decreased dramatically with distance, dropping from 124 pmol min^{-1} mg^{-1} in the plume to 68 at 1.8 km and to levels normally found at reference sites by 3.3 km from the mouth of Blackbird Creek (Table 1). Both white sucker and lake whitefish were only collected from two sites in Jackfish Bay, but neither species showed a decline in activity levels between sites (Table 1).

Mill shutdown occurred from September 12 to 20, and given the 8 to 10 d retention time in the secondary treatment lagoon and the 2 d travel time in Blackbird Creek, the fish in Jackfish Bay were not exposed to fresh effluent for at least a 2 wk period prior to collection (September 27 to 30). This was consistent with visual observations at the foam barrier across the mouth of Blackbird Creek, where the amount of foam, colour, flow and the size of the plume increased on September 29 relative to September 28. Longnose sucker showed no evidence of EROD induction during the September collections, while whitefish EROD activity declined to reference levels at Little Nick rock (1.8 km) and white sucker showed a 40% drop in activity at the plume site (Table 1). No white sucker were captured at Little Nick rock in September. However, white sucker were collected from Cody Island, 1.3 km to the southwest of Blackbird Creek (Table 1) where activity was high (105.3 ± 29.5 (10) pmol min^{-1} mg^{-1}).

Relative to the Black Bay reference site, plasma sex steroid levels were depressed in female longnose sucker during September (Figure 3) and in female white sucker during August and September (Figure 4); samples were not collected from longnose sucker during August. Testosterone levels were lower in female longnose sucker at all Jackfish Bay sampling locations, including 5.5 km from Blackbird Creek ($p=0.006$), although levels of 17β-estradiol were only significantly depressed at the site closest to Blackbird Creek ($p=0.045$). There were no differences between sites in male testosterone levels in longnose sucker during September ($p=0.71$). White sucker females exhibited lower 17β-estradiol levels during both August ($p=0.006$) and September ($p<0.001$), while testosterone depressions were not statistically significant ($p.>0.20$). White sucker male testosterone levels were not significantly different between sites in August ($p=0.17$), but were significantly higher at the BKME site in September ($p=0.03$).

There were no differences in size, liver weight or gonadal weight of longnose sucker with distance from Blackbird Creek or when compared to the reference site, although BKME fish both males ($p=0.03$) and females ($p=0.001$) were considerably older (Table 2). Male white sucker at the plume site showed an increase in liver size in both August and September ($p<0.001$) and a decreased gonad size in August only ($p<0.001$). Female white sucker exhibited a larger liver size in September ($p=0.02$) and a smaller gonad size in both August ($p=0.01$) and September (<0.001) (Table 3). Regressions of length versus adjusted body weights (log-transformed) showed that both male and female white sucker ($p<0.001$), and male longnose sucker ($p=0.005$) were shorter

and heavier than fish from the reference site in September ($p<0.001$); during August this was true for male white sucker ($p<0.001$) but not for females ($p=0.052$).

DISCUSSION

White sucker collected from Jackfish Bay during primary treatment of the BKME showed a marked elevation in MFO activity as measured by B(a)P and PPO metabolism, as well as EROD activity. Studies conducted during August 1990 at Jackfish Bay did not demonstrate any decline in MFO activity in white sucker following the initiation of secondary treatment in October 1989. MFO activity was elevated relative to reference fish in all four species examined in 1990, although there was considerable variation between species in levels of activity and relative induction.

Longnose sucker exhibited the highest activity in the BKME plume, but activity was also highest in this species at both reference sites, and their activity declined fastest with distance from the mouth of Blackbird Creek. The decline in EROD activity with distance from the effluent varied dramatically between species. Between 0.7 and 1.8 km from the effluent, longnose sucker EROD activity declined almost 50%, while white sucker EROD activity did not change appreciably. Between 1.8 and 3.3 km, longnose sucker EROD activity in August declined from 9-fold induction to levels normally found at reference sites, while lake whitefish levels remained relatively constant (6.8-fold and 4.7-fold induction, respectively). Species differences in EROD response at the same site have been reported previously [22]. The reasons for the differences in response in the present study are unknown. Lake whitefish and lake trout showed lower absolute activities, and both species are fall spawners, while both sucker species are spring spawners. Although seasonal changes in MFO activity are well known [4,19,23-25], the changes are thought to occur close to spawning time; August samples were collected at least two months prior to spawning.

The absence of overlap in EROD activity levels in longnose sucker collected at adjacent sites in August suggests that this species is relatively sedentary, since the sites were separated by less than 1 km. The uniformity of induction in lake whitefish between Little Nick and St. Patrick suggests that this species is more mobile, and that the zone of induction is limited to an area between Little Nick and St. Patrick. The dominant current in Jackfish Bay is counter-clockwise,

due to the prevailing westerly winds. This is consistent with the higher level of EROD activity in white sucker collected near Cody Island. Calculation of exposure levels is difficult, due to increased surface temperatures associated with the effluent, the presence of a strong thermocline in summer months and the influence of winds on mixing and distribution of the surface water plume. Based on data collected in 1987 and 1988 (K. Sherman, Ontario Ministry of Environment, Toronto, ON, Canada M4V 1P5; unpubl. data), surface dilutions are estimated to be 5:1 at the plume station, 15:1 at Little Nick and >100:1 at St. Patrick. The plume, however, is only present in the top 3 to 4 m of water; fish were captured at depths of 6 to >30 m. Total organic carbon (TOC) values in the effluent prior to secondary treatment ranged from 184-318 µg/mL TOC [15], while sediment TOC levels range from 400 µg/g at the mouth of Blackbird Creek, to 50-60 at the plume station, 35-40 at Little Nick and 30 at St. Patrick I. (Sherman, unpubl. data). These TOC levels correspond to levels which are 5.4% (at the plume station), 1.4% (at Little Nick) and <0.1% (at St. Patrick) of TOC input associated with the BKME (relative to the mouth of Blackbird Creek).

Samples collected two weeks after a planned mill maintenance shutdown showed no evidence of EROD induction in longnose sucker, reduced activity in white sucker in the plume and a marked reduction in the size of the impact zone for lake whitefish. These data suggest that the duration of MFO induction is relatively short-lived after cessation of exposure, and that secondary treatment has not been successful in removing the compounds responsible for inducing MFO activity in wild fish. If induction was related to historical sediment contamination, it would have persisted through the shutdown period. The decline in white sucker EROD activity with shutdown is consistent with caging studies conducted during August 1989; white sucker moved from the effluent to clean water for 4 d showed a decline in PPO activity from 22.57 ± 4.06 (6) (mean ± SE (n)) to 9.12 ± 1.49 (9) FU min^{-1} mg^{-1} protein and a decline in BaP activity from 1275.5 ± 169.5 to 636.0 ± 86.0 pmol 3-OH B(a)P min^{-1} mg^{-1} [33]. The reduced activity levels in September longnose sucker were not due to the movement of new fish into the bay during shutdown. Steroid reductions in female longnose sucker, consistent with those reported in both white sucker [4,12] and lake whitefish [13] during mill operation, were found at all sites examined during September. Although this study only measured testosterone and 17β-estradiol, previous studies have shown similar impairments in 11-ketotestosterone and 17α20β-dihydroxy-4-pregnen-3-one [12]. These steroid abnormalities appear to be very persistent, and are still evident in white sucker during spawning [12, Munkittrick and Van Der Kraak, unpubl. data], despite the movement of the fish into

uncontaminated spawning areas and the absence of MFO induction [4,12].

Induction of hepatic MFO activity in Jackfish Bay species varied from 8 to 20-fold and disappeared or declined in all three species examined during a mill operational shutdown. The induction levels in both sucker species are equal to, or greater than EROD inductions previously reported after exposure to BKME, except for levels in yearling coho salmon (Oncorhynchus kisutch) collected in the Fraser River (Table 4). Dioxins and furans, and dioxin-like compounds have been shown to induce MFO activity, and have been suggested as factors responsible for increasing MFO activity downstream of bleached kraft mills [6,7], but laboratory studies conducted with 2,3,7,8-substituted dioxins and furans suggest that MFO induction associated with these compounds is long-lived. Induction by i.p. injection of 2,3,7,8- tetrachlorodioxin (TCDD) which ranged from 6 to 16-fold at 6 wk [34] showed no decline in EROD activity after 12 wk of depuration [35]. Muir et al. [18] found that EROD induction associated with dietary exposure to 2,3,4,7,8-pentachlorodibenzofuran (PnCDF) persisted at least 180 d after the cessation of exposure, even though the half-life for elimination of the PnCDF was 61 to 69 d. The half-life of EROD activity was estimated to be 113 d [18]; a long half-life has also been obtained for 2,3,7,8-tetrachlorodibenzofuran (D. Muir, Dept. Fisheries and Oceans, Winnipeg, MN, Canada R3T 2N6, unpubl. data).

Hahn et al. [36] found TCDF induction of P450IA1 mRNA levels to be sustained after 14 d, whereas fish induced with β-napthoflavone reach reference levels within 2 to 6 d [37,38]. EROD activity at low doses of β-napthoflavone also returns to reference levels within 6-8 d at low doses (<1 mg kg^{-1}) [39] and within 14 d at high doses (100 mg kg^{-1}) [40]. The inducing agents from the BKME in Jackfish Bay appears to be acting similar to the β-napthoflavone-type induction, rather than the TCDF/TCDD-type of long-lasting induction. This would suggest that the inducing agents are rapidly cleared, and are not higher-chlorinated dioxins or furans. The pharmacokinetics of many low molecular weight components of BKME are unknown but if they have low chlorine substitution and contain alkyl- and/or hydroxyl- substituents, they are likely to be cleared rapidly [41]. Opperhuizen and Sijm [42] suggest that di-, tri- and non-2,3,7,8- substituted PCDD/Fs are rapidly cleared by fish after waterborne or dietary exposures. Similar rapid clearance rates have been reported for chlorophenols, chloroguiacols [43] and resin acids [Niimi, A.J. Dept. Fisheries and Oceans, Burlington, Ontario L7R 4A6; unpubl. data], although these compounds have not

been associated with EROD induction. The identity of the inducing agents remains unknown, but at Jackfish Bay, these agents appear to be rapidly cleared by fish, water soluble and are not removed by secondary treatment. EROD induction has also been reported in white sucker downstream of another BKM with secondary treatment [5].

Both female longnose and white sucker showed reduced levels of 17β-estradiol and testosterone, similar to findings for lake whitefish collected during August, 1990 [13]. Despite the low levels of steroids in August, significant differences in gonadal weights are present in white sucker at this time of the year [4,12, present study] and testosterone levels were reduced in 1988 [4] and 1989 [12]. The low steroid level do not appear to be related to gonad size, since longnose sucker show lower steroid levels but do not show difference in gonad size. It is not known whether the absence of changes in male longnose sucker steroid levels was due to the shutdown, to a decreased sensitivity of male longnose sucker to BKME, or to poor suitability of testosterone as an early developmental indicator in longnose sucker. Levels of testosterone in spring spawning fish, such as longnose and white sucker, are generally very low in July (<300 pg mL^{-1}) and August (<1000 pg mL^{-1}), and rise to much higher levels prior to spawning (7000 to 15000 pg mL^{-1}) [12]. The persistence of the reduction in gonadal size in male white sucker, similar to previous years, suggests that testosterone may not be the best indicator for male fish during early gonadal development. This study only examined two sex steroids, levels of testosterone, 11-ketotestosterone, 17β-estradiol and 17α20β-dihydroxy-4-pregnen-3-one have been shown to be reduced in white sucker exposed to BKME in Jackfish Bay, relative to Mountain Bay and Black Bay [11,12] as well as inland reference lakes [4,12]. Earlier studies on Jackfish Bay white sucker have detected differences in 11-ketotestosterone and gonadal size during early development, when differences in testosterone were not evident [12].

In longnose sucker, MFO induction in August was only evident up to 1.8 km from the mouth of Blackbird Creek, and no MFO induction was evident in September. Steroid reductions in female longnose sucker persisted to at least 5.5 km off the mouth of Blackbird Creek, at a time when MFO levels were not induced. This suggests that sex steroid problems in females are more persistent than MFO induction, and that MFO induction may be independent of the declines in circulating steroid levels. All changes evident in the white sucker and lake whitefish populations, such as reduced gonadal size, changes in fecundity and egg size, increased age to maturity,

decreased secondary sexual characteristics [12,13] and altered lipid metabolism can be correlated with changes in steroid levels. The reduced levels of circulating steroids appear to be related to reduced steroid synthetic ability and an inability, or reduced ability, of the hypothalamic-pituitary-gonadal axis to respond to alterations in sterold levels [44]. It is unknown if the persistence of these steroid abnormalities in Jackfish Bay is related to the presence of food chain contamination associated with historical contamination, or whether secondary treatment has not removed the responsible chemicals.

Summary

The installation of secondary treatment, along with other process changes have reduced daily effluent discharges of TSS, BOD, phosphorus and total chlorine, as well as eliminated acute lethality, improved water clarity and reduced the temperature of the discharge substantially. Persistence of the induction of hepatic MFO activity after secondary treatment, and the disappearance of MFO induction during a maintenance shutdown at the mill, suggests that impacts are not related to historical sediment contamination, that secondary treatment has not been successful in removing "MFO-active" compounds from BKME and that whatever is causing elevated MFOs is rapidly cleared by fish. The depression of testosterone in female longnose sucker observed 5.5 km from the mouth of Blackbird Creek suggests that steroid changes are more persistent than MFO induction, and that steroid changes and MFO induction are not directly related. The absence of an impact of depressed steroid levels on gonadal size in longnose sucker suggests that steroid changes can be used as a biomarker below levels of contamination exerting effects on MFO activity or gonadal development.

Acknowledgements

MFO measurements were conducted by Lynne Luxon (DFO, Burlington) and assisted by John Forbes and John Hutchinson. Funding for this study was provided by both the Department of Fisheries and Oceans and the Ontario Ministry of Environment Research Advisory Council (Grant

463G and 494G). The assistance of Ian Smith and Dave Rokosh (Water Resources Branch, OME, Rexdale, Ontario) was valuable. Field collections were supervised by Leo Marchand (Marchand Fisheries), and the assistance of Linda Melnyk-Ferguson (Ontario Ministry of Natural Resources, Terrace Bay, Ontario) and Lorne Townes (Ontario Ministry of Natural Resources, Nipigon, Ontario) are gratefully acknowledged. The authors would like to acknowledge the earlier reviews of Drs. D. Muir, S. Brown, A. Niimi, as well as Ian Smith and two anonymous reviewers who helped in the organization of the manuscript. The views and ideas expressed in this manuscript are those of the authors and do not necessarily reflect the views and policies of the provincial or federal government, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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Table 1. Hepatic ethoxyresorufin-o-deethylase activity (pmol min^{-1} mg^{-1} protein) in fish collected at reference sites and at various distances from the mouth of Blackbird Creek where the bleached kraft mill effluent reaches Lake Superior. Samples were collected during 1990, after the initiation of secondary treatment of the effluent (September, 1989) and values reported are for pooled sexes, with the exception of plume white sucker females in August. Values are reported as mean ± SE (n) and values within a row sharing an alphabetical superscript are not significantly different.

	MONTH	REFERENCE SITE[1]	BKME-Exposed[2] PLUME	LITTLE NICK	ST. PATRICK	PEBBLE BEACH	MARATHON[3]
LONGNOSE SUCKER	AUG	7.6±2.3 (6)^{A}	123.9±23.4 (12)^{B}	68.0± 9.7 (13)^{B}	8.1±2.2 (15)^{A}	-	25.7±7.3 (5)^{C}
	SEPT	11.2±3.9 (11)^{A}	4.8± 1.1 (11)^{A}	8.4± 3.4 (3)^{A}	6.6±1.8 (12)^{A}	3.1±0.8 (12)^{A}	-
LAKE WHITEFISH	AUG	1.7±0.4 (10)^{A}	-[4]	11.5± 1.8 (23)^{B}	8.0±1.2 (4)^{B}	-	-
	SEPT	3.4±0.8 (4)^{A}	11.8± 5.6 (4)^{B}	1.9± 0.5 (3)^{A}	-	-	-
WHITE SUCKER	AUG	3.5±0.5 (11)^{A}	72.5± 9.5 (31)^{B5}	79.4±12.8 (15)^{B}	-	-	-
	SEPT	3.0±0.5 (21)^{A}	51.7± 9.0 (19)^{B}	-	-	-	-
LAKE TROUT	AUG	-	-	21.2± 3.4 (13)^{A}	-	-	3.2±1.5 (6)^{B}

[1] Reference site for August collection was Mountain Bay; reference site for the September collection was Black Bay.

[2] Distances from the mouth of Blackbird Creek: plume 0.7 km; Little Nick rock 1.8 km; St. Patrick Island 3.3 km; Pebble Beach 5.2 km. The mouth of Blackbird Creek is approximately 15 km from the discharge point of the mill into Blackbird Creek.

[3] Collections made outside of Peninsula Harbour, where fish are exposed to the BKME from a mill in Marathon.

[4] No collection made

[5] females only, male ERODs significantly larger than females only at this site; value for pooled sexes was 83.4 ± 8.7 (44)

Table 2. Characteristics of longnose sucker collected during September, 1990. Numbers within a sex sharing an alphabetical supercript were not significantly different.

Sex	Site	Distance[1] (km)	Age (y)	Length (cm)	Weight (g)	Liver Weight (g)	Gonad Weight (g)
Male	Plume	0.7	18.0 ± 5.0(4)^{A}	39.6 ± 2.5(5)^{A}	923 ± 197(5)^{A}	14.4 ± 7.3(5)^{A}	58.3 ± 40.8(5)^{A}
	St. Patrick	3.3	17.7 ± 4.7(6)^{A}	40.9 ± 1.9(6)^{A}	912 ± 141(6)^{A}	11.2 ± 1.4(6)^{A}	64.7 ± 16.6(5)^{A}
	Pebble Beach	5.2	17.3 ± 3.5(7)^{A}	40.4 ± 1.2(7)^{A}	942 ± 61(7)^{A}	13.1 ± 2.1(7)^{A}	67.4 ± 7.9(7)^{A}
	Reference	Ref	12.5 ± 1.2(4)^{B}	40.5 ± 3.7(4)^{A}	890 ± 206(4)^{A}	14.8 ± 5.4(3)^{A}	67.2 ± 21.9(4)^{A}
Female	Plume	0.7	14.7 ± 3.3(6)^{a}	43.0 ± 1.2(6)^{a}	1064 ± 71(6)^{a}	13.4 ± 2.9(6)^{a}	91.7 ± 18.3(6)^{a}
	St. Patrick	3.3	15.2 ± 3.4(6)^{a}	41.7 ± 2.4(6)^{a}	955 ± 206(6)^{a}	15.1 ± 5.8(6)^{a}	75.4 ± 44.4(6)^{a}
	Pebble Beach	5.2	16.5 ± 3.0(6)^{a}	42.0 ± 1.8(6)^{a}	1071 ± 135(6)^{a}	19.4 ± 5.6(6)^{a}	89.0 ± 21.8(6)^{a}
	Reference	Ref	10.0 ± 1.0(7)^{b}	42.1 ± 2.8(7)^{a}	1015 ± 221(7)^{a}	15.8(1)	89.3 ± 38.6(7)^{a}

[1] Distance from the mouth of Blackbird Creek.

Table 3. White sucker characteristics during August and September, 1990. Numbers within a month sharing an alphabetical supercripts were not significantly different (sexes analyzed separately).

Sex	Month	Site	Age (y)	Length (cm)	Weight (g)	Liver Weight (g)	Gonad Weight (g)
Male	August	Plume	8.8±0.6 (19)^{A}	38.9±0.5 (20)^{A}	910±33 (20)^{A}	20.2±2.7 (15)^{A}	37.2±3.6 (16)^{A}
		Reference	12.3±1.1 (15)^{A}	41.4±0.4 (15)^{B}	963±24 (15)^{A}	12.7±0.6 (14)^{B}	64.5±5.0 (15)^{B}
	Sept.	Plume	11.4±0.7 (12)^{a}	39.4±0.4 (13)^{a}	969±34 (13)^{a}	20.0±2.0 (11)^{a}	42.2±3.3 (13)^{a}
		Reference	12.7±0.6 (19)^{a}	40.0±0.3 (19)^{a}	864±14 (19)^{b}	12.2±0.9 (18)^{b}	47.6±2.0 (19)^{a}
Female	August	Plume	10.3±0.4 (41)^{C}	41.6±0.3 (41)^{C}	1015±25 (41)^{C}	19.4±1.6 (18)^{C}	27.4±1.4 (23)^{C}
		Reference	9.3±0.9 (15)^{C}	42.7±0.7 (15)^{C}	1042±43 (15)^{C}	17.0±1.0 (15)^{C}	33.9±2.2 (15)^{D}
	Sept.	Plume	11.0±0.3 (37)^{e}	40.8±0.3 (37)^{e}	1039±19 (37)^{e}	23.4±1.0 (37)^{e}	56.8±2.9 (37)^{e}
		Reference	10.9±0.4 (20)^{e}	42.0±0.3 (20)^{d}	1015±20 (20)^{e}	19.3±0.9 (19)^{d}	82.2±3.0 (20)^{d}

Table 4. Comparison of EROD activity (pmol min^{-1} mg^{-1}) of fish exposed to bleached kraft pulp mill effluent.

Species		Location	Exposed	Reference	Induction	Citation
White sucker	Catostomus commersoni	wild	83.4	3.5	23.8	present study
Longnose sucker	Catostomus catostomus	wild	123.9	7.6	16.4	present study
Lake whitefish	Coregonus clupeaformis	wild	11.5	1.7	6.8	present study
Whitefish	Coregonus muksun	caged in field (21 d)			11.5	9
Roach	Rutilus rutilus	wild	23.9	1.1	21.7	22
Perch	Perca fluviatilis	wild	11.7	15.5	0.8	22
		wild	450-600	<100	4.5-6	26
		wild	400-700	<50-100	5-20	27
Fourhorn sculpin	Myoxocephalus quadricornis	lab (9 months to 0.6% BKME)	450	100	4.5	28
Bream	Abramis brama	wild	50.9	7.7	6.6	22
Chinook salmon	Oncorhynchus tshawytscha	wild	430-770	14-43	55	7
Rainbow trout	Salmo gairdneri	caged in field (21 d)			7	29,30
		lab (14 d to 0.7% effluent)	53.9	48.3	1.1	29
		lab (7 wk to 400 times dilution)	<7	<1	7	31
		lab (25 d at 0.5 to 0.75%)	49.1	41.1	1.2	32

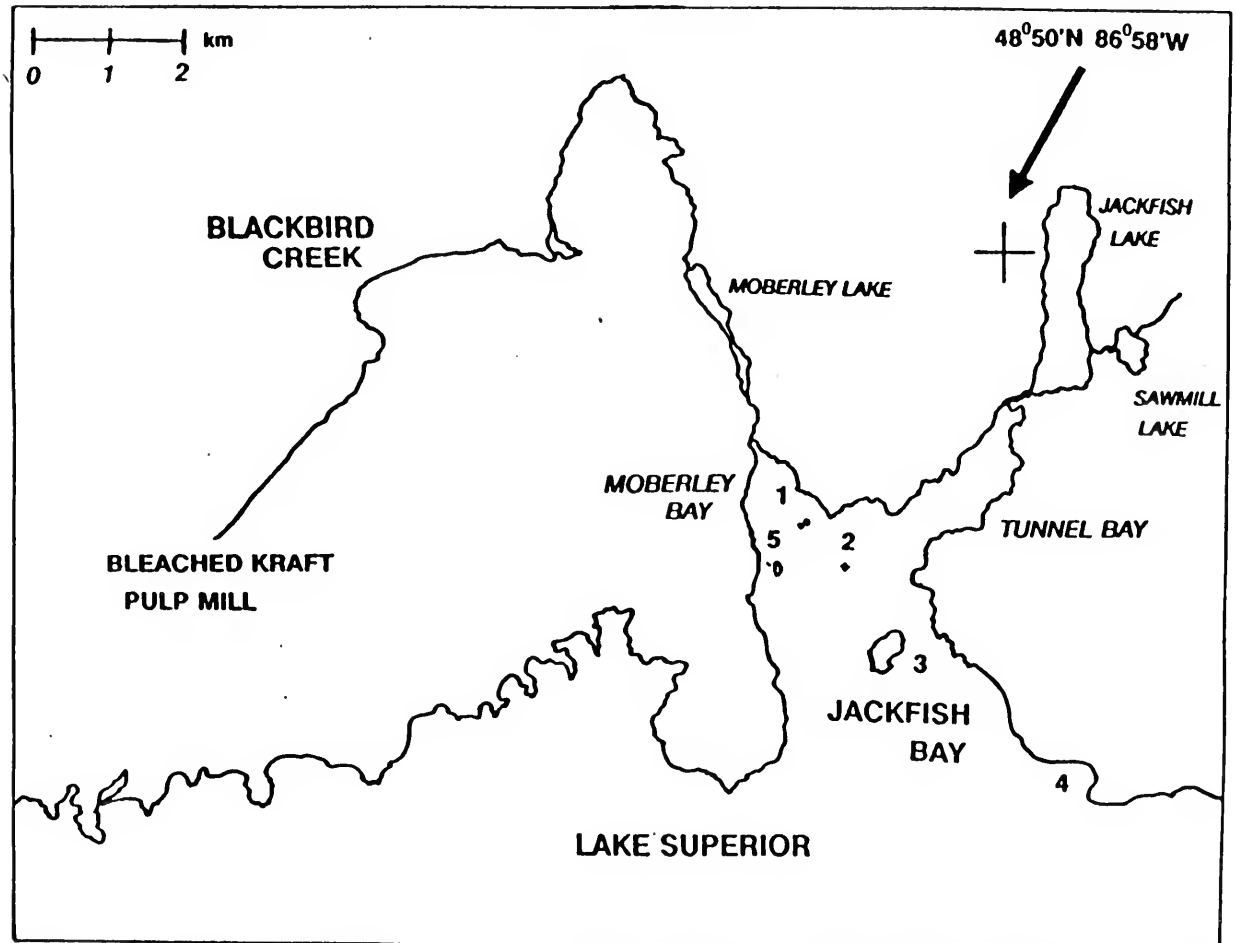
Legend to Figures

Figure 1. The Jackfish Bay study site. The effluent enters through Blackbird Creek and fish were collected at a (1) plume site, (2) Little Nick Island, (3) St. Patrick Island, (4) Pebble Beach and (5) Cody Island.

Figure 2. MFO activity in white sucker prior to (1988 and 1989), and after secondary treatment (1990) of effluent. Solid bars represent values at a reference site (Mountain Bay), cross-hatched bars represent values from Jackfish Bay, and shaded bars represent induction (exposed/reference). All samples were collected during August. Measurements for B(a)P are expressed as Fluorescence Units (FU) min^{-1} mg^{-1} for 1988 samples, pmol 3-OH B(a)P min^{-1} mg^{-1} protein in 1989 and pmol of 3-OH ^{3}H-B(a)P min^{-1} mg^{-1} in 1990; PPO are expressed as FU min^{-1} mg^{-1}, and EROD activity levels are expressed as pmol resorufin min^{-1} mg^{-1}. Values represent measurements for only females (n= 5 or 6 for all samples except EROD for 1989 where n=3-5); values for 1988 and 1989 B(a)P and PPO are from [4,12], while 1990 B(a)P are from Metner and Lockhart (unpubl.).

Figure 3. Plasma levels of gonadal sex steroids (testosterone at BKME site as solid bars; 17β-estradiol as shaded bars) in longnose sucker collected during September, at various distances from the mouth of Blackbird Creek (1=plume, 2=St. Patrick, 3=Pebble Beach). An asterisk denotes a signficant difference ($p<0.05$) from the reference site.

Figure 4. Plasma levels of gonadal sex steroids (testosterone at BKME site as solid bars; 17β-estradiol as shaded bars) in white sucker collected in the plume during August and September. An asterisk denotes a signficant difference ($p<0.05$) from the reference site.


km
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1
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48°50'N 86°58'W
BLACKBIRD CREEK
MOBERLEY LAKE
JACKFISH LAKE
SAWMILL LAKE
MOBERLEY BAY
TUNNEL BAY
BLEACHED KRAFT PULP MILL
1
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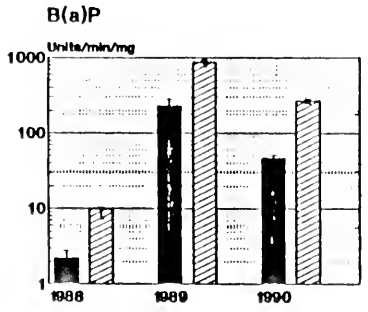
5
JACKFISH BAY
LAKE SUPERIOR


B(a)P
Units/min/mg
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1988
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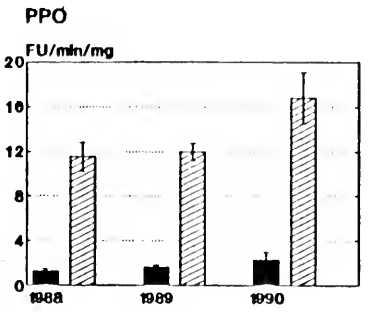
PPO
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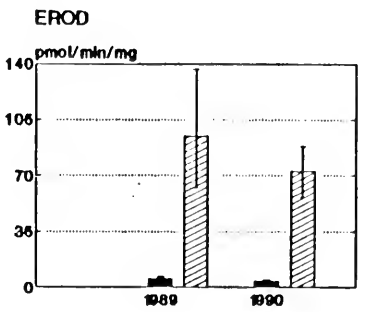
EROD
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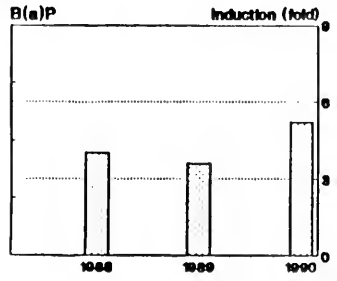
B(a)P
Induction (fold)
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3
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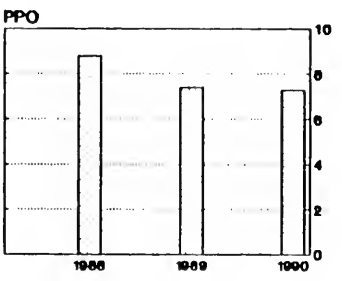
1990


PPO
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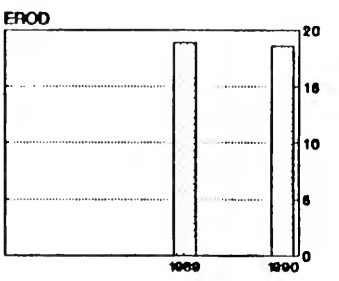
EROD
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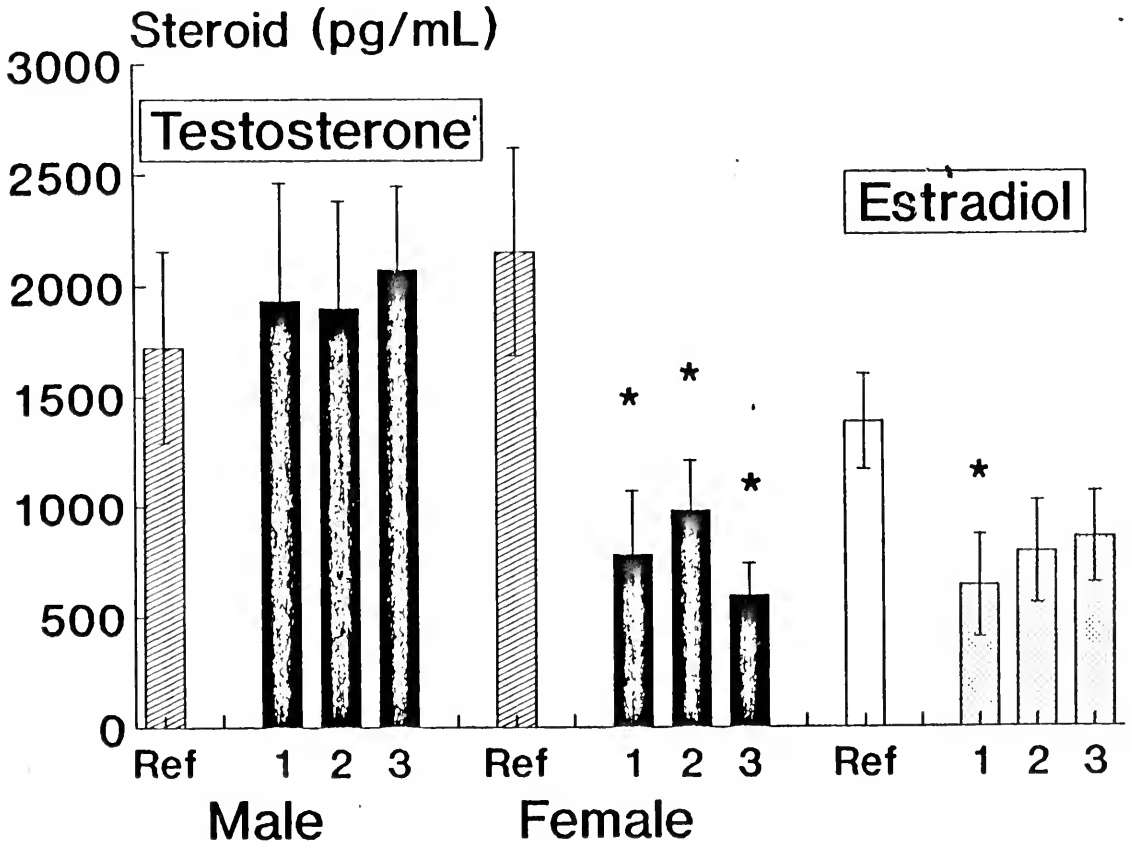
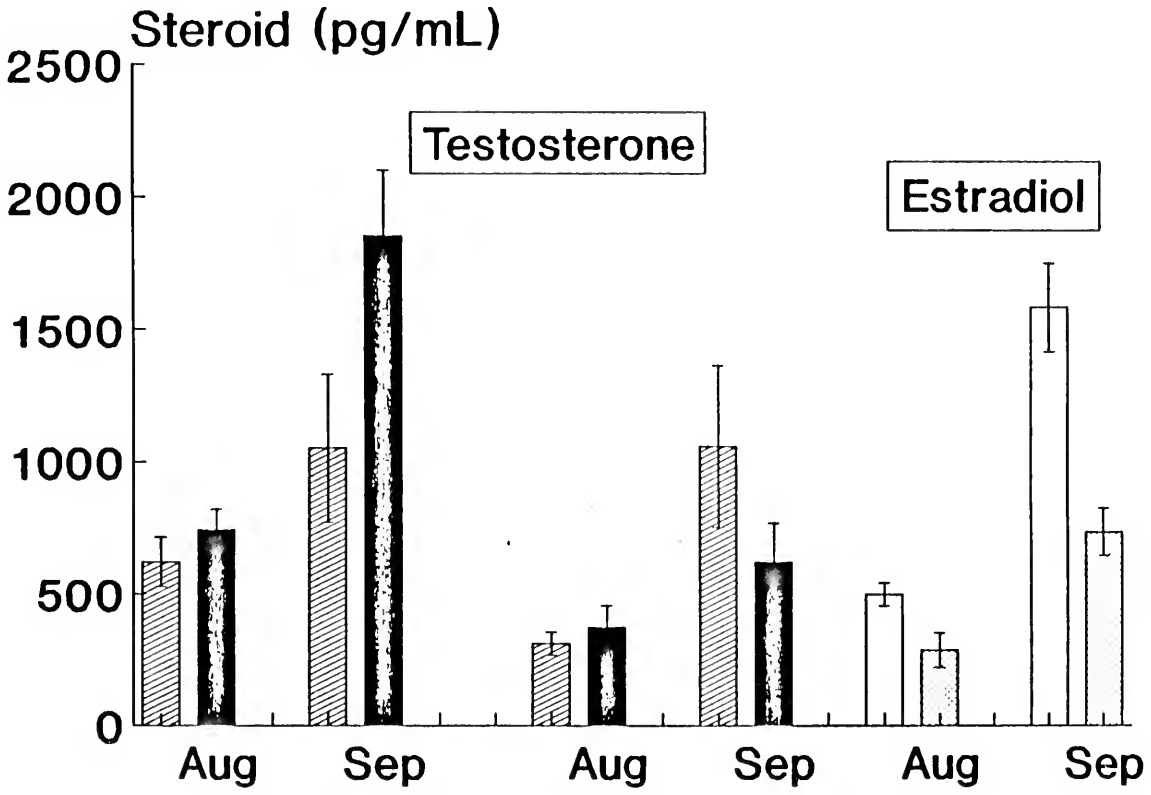
1989
1990

Longnose sucker



White sucker



BLEACHED KRAFT PULP MILL EFFLUENT (BKME) ALTERS STEROID PRODUCTION, REGULATION AND METABOLISM IN WHITE SUCKER (*Catostomus commersoni*)

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Summary

This study examined the basis of reproductive dysfunction in white sucker exposed to effluent from a 1200 tonne d^{-1} bleached kraft pulp mill discharging into Jackfish Bay, Lake Superior. These studies demonstrated that BKME exposure impacts multiple sites within the pituitary-gonadal axis, including depressed pituitary responsiveness to sGnRH, depressed steroidogenic capacity, and altered peripheral metabolism of circulating steroids.

Introduction

Our previous studies have demonstrated reproductive dysfunction in white sucker, longnose sucker (*C. catostomus*) and lake whitefish (*Coregonus clupeaformis*) exposed to BKME [1,2]. The dysfunctions include delayed sexual maturity, altered fecundity, reduced secondary sexual characteristics and depressed circulating sex steroids (testosterone [T], 11-ketotestosterone, 17α,20β-dihydroxy-4-pregnen-3-one [17,20β-P] and 17β-estradiol). All three species show a marked induction of hepatic mixed function oxygenase (MFO) activity (8-20 fold increase), coincident with the decreased levels of circulating steroids. This study examined the basis of reproductive dysfunction in prespawning white sucker exposed to BKME.

Methods

White sucker were collected by hoop net during prespawning migrations; BKME-exposed fish ascend an uncontaminated stream, and the period of residency in clean water prior to spawning was unknown. Assessment of reproductive function included measurement a) of steroid response to injection of D-Arg^6, Pro^{9}N-Et sGnRH (0.1 mg/kg); b) *in vitro* steroid production response of ovarian follicles to hCG, and c) measurement of circulating levels of free and glucuronated sex steroids in circulation.

Results

Although BKME-exposed white sucker are capable of spawning viable eggs, sGnRH failed to induce ovulation in preovulatory fish during a 24 h period, while 10 of 10 fish from the reference site ovulated within 6 h. BKME-exposed fish showed lower plasma levels of both T and 17,20β-P at time 0 (Fig. 1a), while no increase in 17,20β-P was seen after injection of the sGnRH. *In vitro* incubations of ovarian follicles revealed depressed basal secretion of T and 17,20β-P and diminished responsiveness to hCG (Fig. 1b). BKME-exposed fish showed lower levels of both free and glucuronated (not shown) T and 17,20β-P in circulation.

Discussion

There are multiple sites within the pituitary-gonadal axis which are impacted by BKME, including depressed pituitary responsiveness to sGnRH, depressed steroidogenic capacity, and altered peripheral metabolism of circulating steroids. PGE_2 production was similar in ovarian follicles from the reference and BKME sites (unpubl. data), suggesting that there is no general impairment of ovarian function, and that the fish are at comparable stages of maturation. The ratio of steroid production between sites is the same as the ratio in blood between sites, suggesting that induced hepatic MFO activity is not associated with altered plasma steroid clearance rates. Independence of hepatic MFO activity and steroidal abnormalities is also suggested by experiments showing a) no change in clearance of injected steroid and b) persistent depression of circulating steroids during spawning and mill shutdown, when MFO levels are not induced. Despite steroidal dysfunctions in white sucker, fertilization, larval development and survival are not impaired [1].

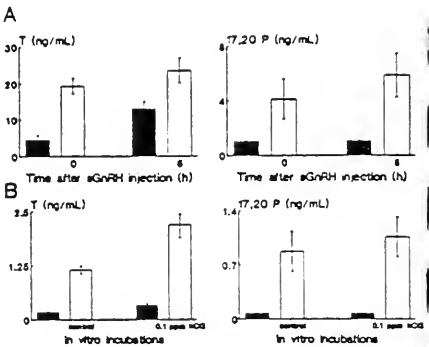

Fig. 1 T and 17,20β-P in white sucker from BKME (closed) or reference (open) sites:
A) plasma of female white sucker after sGNRH injection, and
B) produced by ovarian follicles after hCG simulation.

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Acknowledgement

Funding was provided by Dept. Fisheries & Oceans and Ontario Ministry of Environment (RAC Grant 463G and 494G).