Effects of oil sands related aquatic reclamation on yellow perch (*Perca flavescens*). II. Chemical and biochemical indicators of exposure to oil sands related waters

M.R. van den Heuvel, M. Power, M.D. MacKinnon, and D.G. Dixon

Abstract: Adult yellow perch (*Perca flavescens*) were stocked into experimental ponds designed to emulate possible aquatic reclamation alternatives of the oil sands mining industry. After 5 and 11 months, mixed-function oxygenase (MFO) activity, liver conjugation enzymes, bile polycyclic aromatic hydrocarbon (PAH) equivalents, and plasma sex steroids were measured. Liver MFO activity and bile PAH equivalent concentration were closely correlated and showed the highest levels in the experimental ponds but also demonstrated a gradient of exposure among reference locations. Levels of steroid hormones in fall-captured fish did not show major differences among sites. However, during winter, yellow perch from three sites, including the experimental ponds, showed low levels of sex steroids in both males and females. Multivariate regressions showed no relationship between steroid hormone concentrations and gonad size or fecundity. Similarly, steroid hormones did not parallel the gradient of exposure as measured by MFO and bile PAH metabolites. Gonad size and fecundity also were not directly correlated with the gradient of exposure observed in this study. Although MFO activity and bile PAH equivalents were good indicators of exposure to oil sands related waters, they were not predictive of physiological endpoints, suggesting that the latter were influenced primarily by ecological and not by chemical factors.

Résumé : On a introduit des perchaudes (*Perca flavescens*) dans des étangs expérimentaux pour simuler les opérations possibles de récupération de sables bitumineux dans les milieux aquatiques par l'industrie minière. Après 5 et 11 mois, on a mesuré l'activité des oxygénases à fonction mixte (OFM), les enzymes hépatiques de conjugaison, les équivalents d'hydrocarbures aromatiques polycycliques (HAP) dans la bile et les stéroïdes sexuels plasmatiques. L'activité des OFM hépatiques et la concentration des équivalents HAP dans la bile étaient étroitement corrélées, et ces deux paramètres ont présenté les valeurs les plus élevées dans les étangs expérimentaux, mais ont aussi révélé l'existence d'un gradient d'exposition dans les sites de référence. Les niveaux d'hormones stéroïdes chez les poissons capturés à l'automne n'étaient pas très différents d'un site à l'autre. Cependant, durant l'hiver, les perchaudes mâles et femelles de trois sites, notamment des étangs expérimentaux, renfermaient de faibles concentrations de stéroïdes sexuels. Les régressions multivariées ont montré qu'il n'y avait pas de relation entre les concentrations d'hormones stéroïdes et la taille des gonades ou la fécondité. De même, les concentrations de stéroïdes ne suivaient pas le gradient d'exposition tel que mesuré par les OFM et les métabolites des HAP dans la bile. La taille des gonades et la fécondité n'étaient pas non plus directement corrélées avec le gradient d'exposition observé dans cette étude. Si l'activité des OFM et les équivalents HAP biliaires étaient de bons indicateurs de l'exposition aux eaux des sables bitumineux, ils ne permettaient pas de faire des prévisions concernant les paramètres physiologiques, ce qui laisse penser que ces derniers étaient déterminés principalement par des facteurs écologiques plutôt que par des facteurs chimiques.

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Introduction

The oil sands industries of northern Alberta, Canada, are the largest producers of synthetic crude oil in the world, with over a billion barrels produced to date. This large and economically important industry will mine hundreds of square kilometres of muskeg-dominated northern boreal forest in order to remove the hydrocarbon-saturated sands located beneath the surface. The oil sands industries have a commitment to reclaim the landscape disturbed by the min-

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ing operations. Out of necessity, this will involve reincorporating the tailings back into the landscape in an environmentally benevolent fashion.

Regardless of the method of reclamation used, surface waters will be produced or influenced by process activities and the resulting waters must be capable of supporting biological systems in viable ecosystems. The water quality properties associated with the process affected waters include elevated salinity and organic acids (naphthenic acids) as well as the increased concentrations of polycyclic aromatic hydrocarbons (PAH's). The relative concentrations of the chemical constituents will differ, depending on the reclamation methods used. It is expected that fish will eventually come into contact with oil sands reclamation waters or inhabit reclaimed aquatic systems. As such, it would be useful to have a suite of biochemical and chemical indicators of exposure to families of compounds associated with oil sands related waters.

Two indicators, mixed-function oxygenase (MFO) activity and bile PAH equivalent concentration, have been widely used to quantify exposure of fishes to petroleum hydrocarbons (Krahn et al. 1984; Hellou and Payne 1987; Payne et al. 1987; Collier and Varanasi 1991). In fishes, MFO generally refers to a measurement of a single cytochrome P450 isozyme, namely P4501A1 (Payne et al. 1987; Stegeman and Hahn 1995). Synthesis of MFO enzymes can be induced by exposure to many of the compounds that are metabolized by them, including PAH's. In the liver, P4501A1 is responsible for the initial oxidation (phase I) of hydrophobic organic compounds in order to facilitate biliary excretion. Oxidized hydrophobic organic compounds are rendered more water soluble via conjugation with glucuronic acid, glutathione, or sulfate. The conjugative metabolic reactions are referred to as phase II and are catalyzed by enzymes such as uridine 5′-diphosphate-glucuronosyl transferase (UDPGT), glutathione *S*-transferase (GST), and sulfatase. Because biliary excretion is the major route of excretion for PAH's, measurement of PAH metabolites in the bile can provide a sensitive indicator of exposure to PAH's.

Levels of plasma steroid hormones in fishes have been observed to decrease in response to PAH exposure (Thomas 1988; Stein et al. 1991) or petroleum hydrocarbon exposure (Truscott et al. 1983; Johnson et al. 1988; Truscott et al. 1992). Recently, plasma steroid hormones have been extensively used to examine the potential of pulp and paper mill effluents to affect fish reproduction (Munkittrick et al. 1991; Van Der Kraak et al. 1992; McMaster et al. 1996). The main estrogen and androgen in fishes, 17β-estradiol and 11-ketotestosterone, are the primary hormones responsible for gonadal development in females and males, respectively. Decreased levels of these steroid hormones and their common immediate precursor, testosterone, would therefore be expected to indicate the potential for contaminant-induced reduction in gonad size.

Toxicological literature has been flooded with research studying biochemical and chemical indicators of exposure to groups of anthropogenic compounds. For the most part, these parameters have been measured under the contention that they can be used as early-warning signs or predictors of damage at higher levels of ecological organization (National Research Council of Canada 1985). The term "indicator" has been coined to imply that measured parameters predict, or are correlated with, effects at a higher level of biological organization (McCarty and Munkittrick 1996). Attempts to causally link, or validate, indicators for effects at higher levels of biological organization are few (Holdway 1996, 1997; McCarty and Munkittrick 1996).

The purpose of this study was to examine the usefulness of MFO enzymes, conjugation enzymes, bile PAH metabolite concentrations, and plasma steroid hormones in yellow perch (*Perca flavescens*) as markers of exposure to oil sands mining related waters. The secondary aim of this work was to examine the potential of these biochemical and chemical endpoints as predictive indicators of physiological and population-level impacts in subsamples of individuals from yellow perch populations exposed to oil sands derived sources of water.

Materials and methods

Study site description and yellow perch stocking experimental design

Study site descriptions and chemical characteristics of the ponds and lakes used in this study are described in detail in van den Heuvel et al. (1999). Briefly, the main experimental pond, called the Demonstration Pond, was constructed in 1993 as an example of the water-capping reclamation strategy. Demonstration Pond is about 4 ha and contains oil sands mature fine tailings capped with a layer of surface drainage water. Two other experimental ponds located at the mine site, South Bison Pond and Deep Wetland, were constructed ponds without a bottom of fine tailings. Deep Wetland was constructed adjacent to and at the same time as Demonstration Pond. South Bison Pond receives drainage from a reclaimed pasture area and is 8 years older than the other two experimental ponds. Mildred Lake is an altered natural lake on the oil sands lease from which yellow perch were stocked into the experimental ponds. Three additional off-site reference locations were chosen in order to examine yellow perch populations that were completely isolated from any oil sands influence. Sucker Lake is located on the western edge of the Athabasca deposit, while Kimowin Lake and Cowper Lake are located in areas that do not receive any surface water drainage from above the oil sands geological deposit.

Yellow perch were removed from Mildred Lake in the springs of 1995 and 1996 and transferred into the experimental ponds as described in van den Heuvel et al. (1999). Subsamples of yellow perch were captured and sacrificed during September 1995 (hereafter referred to as fall 1995), May 1996 (spring 1996), September 1996 (fall 1996), and March 1997 (winter 1997).

Fish sampling

Yellow perch were captured as described in van den Heuvel et al. (1999). Blood was removed by caudal puncture using a 3-mL syringe (21 gauge or 23 gauge), placed into a 3-mL heparinized vacutainer, and placed on ice. After blood collection, yellow perch were dispatched with a sharp blow to the head. Within 8 h of collection, blood was spun at 500 × *g* for 10 min in a clinical centrifuge and plasma was frozen in liquid nitrogen.

Livers were removed and weighed, and a 1-g subsample for 7-ethoxyresorufin-*O*-deethylase (EROD) analysis was frozen in liquid nitrogen in a 2.0-mL cryovial. Gallbladders that contained bile were placed intact into 2.0-mL cryovials and frozen in liquid nitrogen. In fall 1995 and spring 1996, bile-containing gallbladders were pooled from each site, while in fall 1996 and winter 1997, gallbladders were collected on an individual fish basis. Liver samples for EROD measurement were stored at –80°C pending analy-

sis (maximum storage time of 3 months). All other samples were stored at –20°C until analysis.

EROD analysis

Hepatic MFO enzyme activity was estimated in postmitochondrial supernatant (PMS) as EROD activity using a modification of the fluorescence plate-reader technique developed by Kennedy and Jones (1994). Liver extracts were homogenized in a cryopreservative buffer (0.1 M phosphate, 1 mM EDTA, 1 mM dithiothreitol, and 20% glycerol, pH 7.4) and spun at 9000 × *g* to obtain the PMS. The EROD reaction mixture contained 0.1 M HEPES buffer, pH 7.8 (Sigma Chemical Co., St. Louis, Mo.), 5.3 mM Mg^{2+}, 0.5 mM NADPH (Boehringer Mannheim, Mannheim, Germany), 1.5 μM 7-ER (Sigma), and about 0.5 mg PMS protein·mL^{-1}. The reaction was stopped after 5 min by adding 0.5 mL of ice-cold acetonitrile containing the protein-binding reagent fluorescamine (Lorenzen and Kennedy 1993). Resorufin was determined on a Millipore Cytofluor model 2350 plate-reading fluorometer (530 nm excitation, 590 nm emission). Protein content was estimated from fluorescamine fluorescence (380 nm excitation, 420 nm emission) against bovine serum albumin (fraction V, Sigma).

Conjugation enzyme analysis

Microsomal and cytosolic fractions were prepared from PMS of female yellow perch captured during winter 1997. PMS was centrifuged for 1 h at 90 000 × *g*. The cytosol (supernatant) was removed and the microsomal pellet was suspended in cryopreservative buffer (previously described). Both cytosolic and microsomal fractions were frozen at –80°C until analysis. Measurement of UDPGT activity was measured in microsomal suspensions according to Castren and Oikari (1983). Protein content was determined by a modified fluorescamine fluorescence method (Lorenzen and Kennedy 1993). GST was measured according to Habig et al. (1974) as modified by Anderson et al. (1985).

Bile fluorescence analysis

Yellow perch gallbladders were thawed and the bile expressed from them. Bile was diluted by a factor of 10 with Milli-Q quality water. High-performance liquid chromatography (HPLC) – fluorescence analysis was used to estimate phenanthrene and benzo[a]pyrene equivalents adapted after the methods of Krahn et al. (1984). The HPLC was a Spectra-Physics model SP8100 pump, a Shimatzu RF-10A fluorescence detector, and a Spectra-Physics model SP4270 peak integrator. Bile samples (50 μL) were injected onto a 250 × 4.6 mm C-18 column with 5-μm particle size (Supelco, Mississauga, Ont.) and eluted with a linear gradient of 100% water (0.005% acetic acid) to 100% methanol over 15 min and then held at 100% methanol for an additional 45 min. Areas for all peaks eluting after 7 min of run time were added and compared with peak areas for standards of either phenanthrene (256 nm excitation, 380 nm emission) or benzo[*a*]pyrene (380 nm excitation, 430 nm emission).

Steroid hormone analysis

Steroid hormones were measured according to McMaster et al. (1992). Plasma samples were thawed and steroid hormones were extracted with diethyl ether. The plasma extract from females was analysed for estradiol and testosterone, while that from males was analysed for 11-ketotestosterone and testosterone using standard radioimmunoassay procedures.

Statistics

All statistical analyses were performed using SYSTAT software (Wilkinson 1990). EROD, bile PAH equivalent concentrations, and steroid hormone data were analysed by ANOVA followed by Tukey's test for all possible pairwise comparisons. Yellow perch EROD injection experiment data were analyzed by ANOVA followed by Dunnett's test. Prior to statistical testing, homoscedasticity was tested using Bartlett's test and normality was examined using probability plots. Heteroscedastic or nonnormal data were log transformed and retested prior to performing statistical tests requiring that these assumptions be met. Statistical significance was assessed at the $\alpha = 0.05$ level.

Results

Hepatic enzyme induction

Over the duration of the study, a consistent pattern of hepatic MFO (EROD) induction was observed in yellow perch exposed to oil sands related waters (Fig. 1). These data demonstrated a gradient of EROD activity with yellow perch from Kimowin Lake and Cowper Lake, the off-site reference locations remote from the Athabasca oil sands deposit, having the lowest levels of activity. Intermediate in EROD activity were Mildred Lake and Sucker Lake yellow perch. These reference sites were located on the oil sands deposit area and, in the case of Mildred Lake, at the mine site. Highest in EROD activity were yellow perch from the experimental ponds South Bison Pond, Demonstration Pond, and Deep Wetland. Male and female yellow perch both demonstrated similar trends in EROD activity. The absolute activity was consistently higher in male than in female yellow perch. The maximum magnitude of EROD induction (highest mean divided by the lowest) for males and females, respectively, was 9.6-fold in winter 1997 and 16.2-fold in fall 1996.

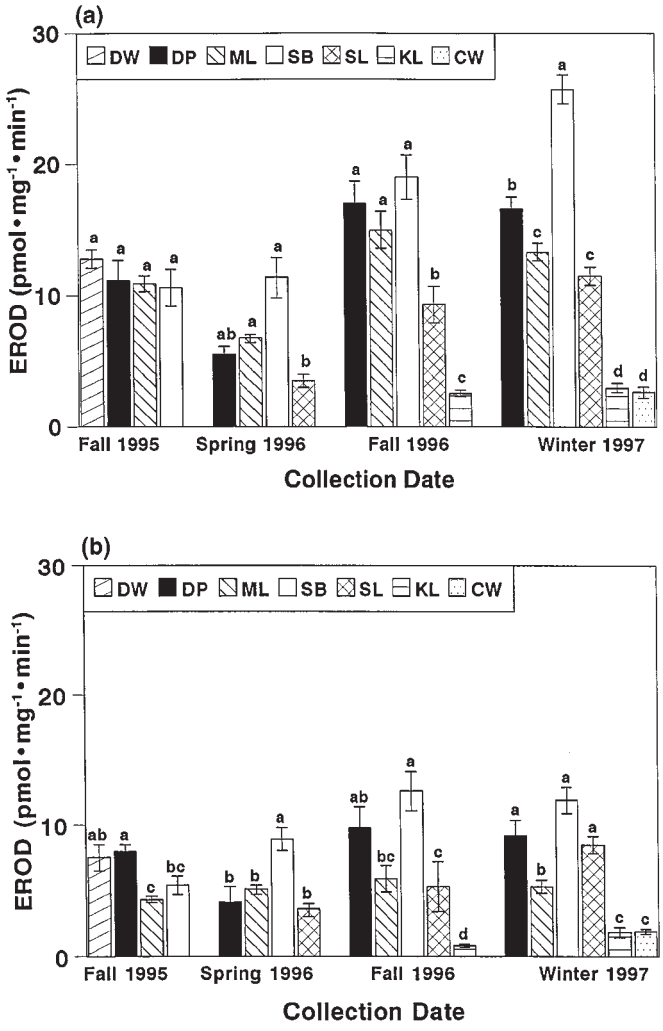
Phase II hepatic metabolism, as represented by GST and UDPGT activity measured in winter 1997, was not affected by exposure to oil sands reclamation related water (Table 1). GST activity in Sucker Lake during this period was shown to be significantly depressed. Due to the lack of changes in these enzyme activities during the winter 1997 period, further analysis on other capture periods was not conducted.

Bile PAH equivalent concentrations

Bile PAH equivalent concentrations measured at both phenanthrene and benzo[*a*]pyrene wavelengths showed similar trends as EROD activity. Due to the low number of individuals with bile present in the gallbladder, male and female yellow perch bile results were pooled for statistical analysis. Differences in both phenanthrene and benzo[*a*]pyrene bile PAH equivalent concentrations were observed for the fall 1996 and winter 1997 sampling periods (Fig. 2). During spring 1996 and fall 1995, bile samples were pooled, precluding statistical analysis, but similar trends were observed. Phenanthrene and benzo[*a*]pyrene endpoints were found to be highly correlated ($r^2 = 0.81$, $p < 0.001$, $n = 93$), indicating a similar source for both groups of compounds.

Multivariate regressions were used in order to determine if bile PAH equivalent concentrations were predictive of EROD activity. Data from individual fish were utilized from fall 1996 and winter 1997. Bile benzo[*a*]pyrene and phenanthrene equivalent concentrations were assessed individually using capture season and yellow perch gender as additional independent variables. There was no significant seasonal component of variation, and capture season was

Fig. 1. Mean ± SEM levels of EROD measured in liver PMS for (*a*) male and (*b*) female yellow perch. Sample sizes in males are $n = 12$ with the following exceptions. Fall 1995: Demonstration Pond, $n = 8$; South Bison Pond, $n = 6$; spring 1996: Demonstration Pond, $n = 4$; South Bison Pond, $n = 2$; fall 1996: Demonstration Pond, $n = 13$. Sample sizes in females are $n = 12$ with the following exceptions. Spring 1996: Demonstration Pond, $n = 4$; Mildred Lake, $n = 10$; South Bison Pond, $n = 8$; fall 1996: Sucker Lake, $n = 13$. Means within a sampling period with the same letter are not significantly different ($p > 0.05$). DW, Deep Wetland; DP, Demonstration Pond; ML, Mildred Lake; SB, South Bison Pond; SL, Sucker Lake; KL, Kimowin Lake; CW, Cowper Lake.



dropped from further analysis. Bile PAH equivalents were highly predictive ($p < 0.001$) of EROD activity when either phenanthrene or benzo[*a*]pyrene endpoints were used. A significant component of variation ($p < 0.05$) could be attributed to gender when phenanthrene equivalents were examined but not when benzo[*a*]pyrene equivalents were used as an independent variable.

Plasma sex steroid hormones

Significant differences in steroid hormones were observed during all capture periods. In females captured during fall sampling periods, only South Bison Pond (in 1995) and Sucker Lake (in 1996) had levels of estradiol that were significantly lower than values measured for the bulk of the other sites (Fig. 3). Testosterone in fall 1995 South Bison Pond female yellow perch was also significantly lower than for two of the other three sites examined. The winter steroid results demonstrated much more dramatic differences. During this sampling period, two discrete groups were observed. South Bison Pond, Demonstration Pond, and Sucker Lake female yellow perch had low plasma levels of both estradiol and testosterone as compared with Kimowin Lake, Cowper Lake, and Mildred Lake yellow perch. This trend was particularly apparent for testosterone where yellow perch from the latter three sites appeared to have increased testosterone levels from the previous fall, while those from the former three sites did not.

Males demonstrated few significant differences in steroid hormone levels in the fall capture periods (Fig. 4). Deep Wetland yellow perch had elevated testosterone concentrations compared with yellow perch from the other three sites examined during fall 1995. There were also significant differences between Kimowin Lake and Mildred Lake yellow perch 11-ketotestosterone in fall 1996. As in females, steroid differences in the late winter were much more dramatic. Identical groupings appeared as in females, with 11-ketotestosterone and testosterone in yellow perch from South Bison Pond, Demonstration Pond, and Sucker Lake being significantly lower than in male yellow perch from the remaining sites.

Comparisons of biochemical and physiological parameters

In order to determine if steroid hormone levels were predictive of physiological reproductive indicators, the reproductive indices gonadosomatic index (GSI) and fecundity somatic index (FSI, data from van den Heuvel et al. 1999) were compared with steroid levels on an individual fish basis using regression analysis. Either GSI or FSI was used as the dependent variable and steroid concentration, capture location, and capture season were used as the independent variables. Regressions showed a strong seasonal component of variability in gonad size in both males and females ($p < 0.001$), but there was no significant seasonal variation in female fecundity. For both estradiol and testosterone in female yellow perch, there were interactions between steroids and location of capture in the GSI–steroid regression and in the FSI–steroid regression ($p < 0.001$). This indicates that there was no consistent relationship between steroids and GSI or FSI within the individual capture locations; the relationship between steroids and GSI or FSI varied from location to location. A similar lack of relationship between gonad size and steroids was observed when mean data were compared. For example, in winter 1997, South Bison Pond female yellow perch had relatively low steroid levels, the largest ovaries, and highest fecundity (van den Heuvel et al. 1999). Conversely, Sucker Lake yellow perch captured during the same period had similarly low estradiol, relatively smaller ovaries, and low fecundity.

In males, there were no interactions between location and steroid levels for either steroid measured. There were also no relationships between steroids and GSI. As can be ob-

Table 1. Mean (SEM, *n*) hepatic activity levels of GST and UDPGT in female yellow perch captured during winter 1997.

Capture location	GST ($\mu mol \cdot mg^{-1} \cdot min^{-1}$)	UDPGT ($pmol \cdot mg^{-1} \cdot min^{-1}$)
Demonstration Pond	1.27 (0.08, 11) *a*	24.1 (4.0, 11) *a*
Mildred Lake	1.40 (0.09, 11) *a*	25.0 (7.6, 11) *a*
South Bison Pond	1.23 (0.09, 12) *a*	27.6 (4.4, 13) *a*
Sucker Lake	0.78 (0.12, 9) *b*	17.4 (3.8, 9) *a*
Kimowin Lake	1.20 (0.12, 10) *a*	18.4 (5.8, 10) *a*
Cowper Lake	1.55 (0.08, 10) *a*	16.0 (7.2, 10) *a*

Note: Means followed by the same letter are not significantly different ($p > 0.05$).

Fig. 2. Mean ± SEM levels of (*a*) benzo[*a*]pyrene and (*b*) phenanthrene equivalent concentrations in the bile of yellow perch as measured by HPLC–fluorescence. Sample sizes for fall 1996 and winter 1997 were $n = 10$. Fall 1995 and spring 1996 bars represent concentrations measured in pooled samples of bile (>10 individuals). Means within a sampling period with the same letter are not significantly different ($p > 0.05$). See Fig. 1 for site abbreviations.

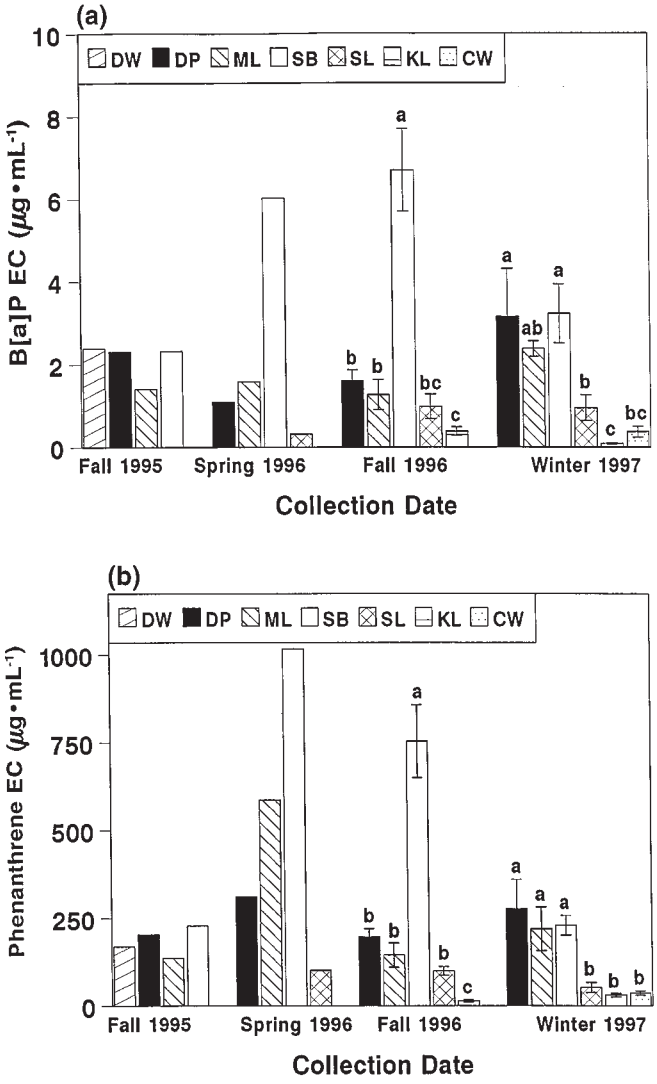
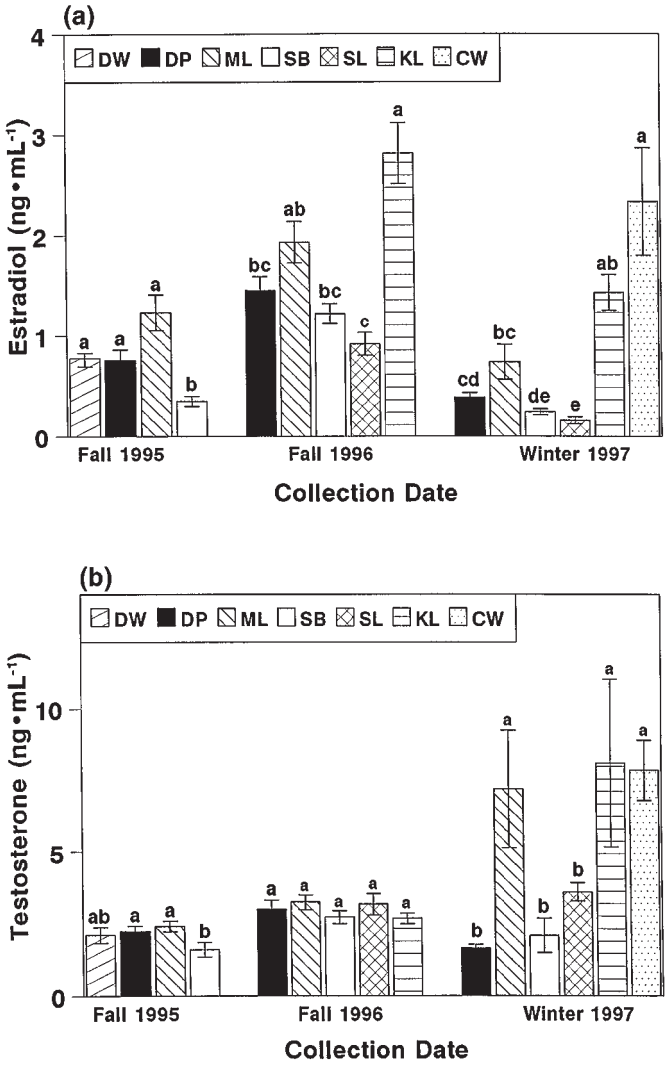


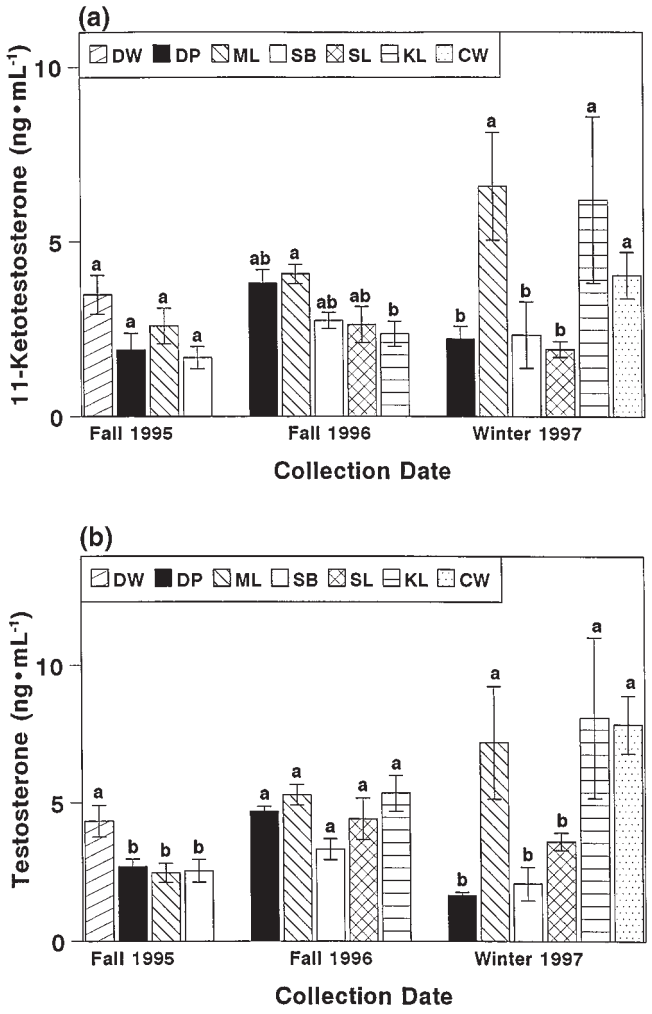
Fig. 3. Mean ± SEM levels of (*a*) estradiol and (*b*) testosterone in plasma of female yellow perch. Sample sizes are $n = 12$ with the following exceptions. Fall 1995: Demonstration Pond, $n = 13$; Mildred Lake, $n = 23$; winter 1997: Mildred Lake, $n = 11$; Sucker Lake, $n = 10$; Kimowin Lake, $n = 11$; Cowper Lake, $n = 9$. Means within a sampling period with the same letter are not significantly different ($p > 0.05$). See Fig. 1 for site abbreviations.



served from the mean steroid levels, there was a significant component of variability due to capture location.

Yellow perch plasma steroid hormones in females did not show the same gradient as the exposure-related indicators EROD and bile PAH equivalent concentrations. Regressions were performed by sex and capture season to determine if

Fig. 4. Mean ± SEM levels of (*a*) 11-ketotestosterone and (*b*) testosterone measured in male yellow perch. Sample sizes are $n = 12$ with the following exceptions. Fall 1995: Demonstration Pond, $n = 8$; Mildred Lake, $n = 13$; South Bison Pond, $n = 6$; winter 1997: Mildred Lake, $n = 10$; Kimowin Lake, $n = 9$; Cowper Lake, $n = 10$. Means within a sampling period with the same letter are not significantly different ($p > 0.05$). See Fig. 1 for site abbreviations.



EROD was predictive of steroid levels. Capture location was also added as an independent variable in the regression. During fall 1995 capture dates, there were significant positive relationships between both 11-ketotestosterone and testosterone and EROD in male yellow perch and also between testosterone and EROD in females. However, during fall 1996, there was a negative relationship between estradiol and EROD in females and between testosterone and EROD in males. During winter 1997, when the most dramatic steroid differences occurred, capture location was a highly significant ($p < 0.001$) determinant of steroid levels, while EROD did not contribute significantly to the overall variability. This trend was apparent for both steroids in both sexes during the winter capture date. Similar regressions using bile PAH equivalent concentrations also showed a lack of any consistent trend between exposure indicators and steroid hormones.

EROD activity and bile PAH equivalent concentrations also could not be related to physiological indices of reproduction. In regressions using GSI as the dependent variable, in both males and females, there was a significant interaction between EROD activity and capture location ($p < 0.001$). This implies that the EROD–GSI relationship varied between capture location. In a regression using fecundity (FSI) as the dependent variable, there was also a significant location effect ($p < 0.001$). No significant interactions and no relationship between EROD and FSI were observed.

Discussion

This study demonstrated a gradient of exposure in yellow perch to oil sands related compounds as indicated by MFO and bile PAH metabolite endpoints; this was not reflected in the conjugation enzyme parameters measured. The highest exposure to the compounds to which these indicators are sensitive appeared to occur in the experimental reclamation related ponds, followed by yellow perch captured in lakes on, or receiving water from, the Athabasca oil sands deposit, and then by yellow perch in lakes located off the Athabasca deposit. Steroid hormones in yellow perch did not follow the same gradient and could not be consistently related to either measure of yellow perch exposure. Steroid hormones, hepatic MFO, and bile PAH equivalent concentrations were not correlated with physiological indices of adult yellow perch reproductive development, gonad size, and fecundity.

Levels of the conjugation enzymes GST and UDPGT did not appear to be related to exposure to water associated with oil sands. The only statistically significant depression occurred with GST in Sucker Lake yellow perch. This may have been a stress-related response due to the hypoxia that the yellow perch were undergoing at the time. Previous studies have identified the GST and UDPGT enzymatic endpoints as potential indicators of exposure to organic compounds (Celander et al. 1994). However, with the species of fish and sources of exposure examined in this study, these endpoints appeared to have no value as exposure indicators.

Hepatic EROD activity measured in experimental yellow perch was likely proportional to the exposure to PAH's originating from oil sands material. P4501A1 induction is indicative of exposure to several groups of planar compounds including dioxins and furans, polychlorinated biphenyls, and PAH's. In this case, the two former classes of compounds are known not to be associated with oil sands mining. The elevated bile PAH equivalent concentrations suggest the presence of PAH metabolites in the bile and thus exposure of these yellow perch to PAH's. Attempts were made to identify particular PAH metabolites in yellow perch bile extract derived from this study. No detectable levels of known PAH metabolites could be identified in the bile (authors' unpublished data). This result may be because the individual metabolites were present at concentrations too low for detection by a mass selective detector. Metabolites of biogenic compounds were also found in the bile in high concentrations, impairing the detection of molecules that may only be present at trace levels.

Exposure to PAH's appears to be diagnostic of exposure to waters associated with oil sands material. In the case of

South Bison Pond, the high responses observed in both MFO and bile PAH equivalent concentrations are almost certainly attributable to unextracted oil sands material, most likely the lean oil sands material that composes the pond basin and underlies the surrounding pasture. Demonstration Pond, which contains large amounts of previously extracted material (tailings), tends to show lower bile equivalent concentrations and hepatic MFO activity than South Bison Pond. In this case, we cannot rule out the possibility that this material is derived from the clays that compose the pond basin rather than from the tailings themselves. Observations from fall 1995 in the adjacent Deep Wetland would lend credence to the theory that the source of at least some PAH-like material is the pond construction material.

The gradient of oil sands related exposure also extends to some of the reference locations. Mildred Lake has obvious sources of this material, since the water pumped into Mildred Lake originates in the Athabasca River, which cuts directly through the Athabasca oil sands deposit. Also, the intake for Mildred Lake water is downstream of a point-source discharge from an adjacent oil sands upgrading plant. Sucker Lake, which is remote from any industry or human activity, also appears to have low-level exposure to what are likely oil sands derived compounds. This was unexpected, since the Athabasca oil sands would be expected to be far below the surface at this location and Sucker Lake is only on the eastern periphery of the deposit. Detectable levels of naphthenic acids in Sucker Lake water suggest that there may be a source of oil sands related compounds present in drainage waters entering the lake (van den Heuvel et al. 1999). Only Kimowin Lake and Cowper Lake appeared to have very low levels of exposure to PAH material. These two sites are completely removed from the Athabasca deposit and do not receive drainage water from any part of the oil sands area.

The exposure gradient observed in this study has important implications for the oil sands reclamation options. These results would indicate that it is essentially impossible to create or reclaim aquatic habitat on the oil sands area without having some exposure to organic (and inorganic) compounds present in the bitumen deposits. Although the indicators of exposure used in this study are specific for particular groups of compounds, PAH's in this case, it is likely that other bitumen-related organic compounds such as naphthenic acids will occur proportionally (they are also proportional to the inorganic compounds associated with oil sands, van den Heuvel et al. 1999). The severity of the exposure to these compounds will depend on the nature of the reclamation material present. It appears, ignoring other characteristics of the material, as if the previously extracted material (tailings) is no worse, and possibly better, than unextracted oil sands as a reclamation material with regard to exposure to compounds to which our exposure indicators were sensitive. This raises the question of what levels of exposure to compounds associated with these materials become detrimental to the integrity of populations of aquatic organisms.

At levels of exposure encountered in this study, no consistent relationships between the level of exposure to oil sands related compounds and steroid hormone concentrations could be found. Observations in rainbow trout (*Oncorhynchus mykiss*) would suggest that levels of estradiol in females during late vitellogenesis should begin to drop gradually, while testosterone levels remain high until ovulation (Scott and Sumpter 1983). It appeared that yellow perch in South Bison Pond, Demonstration Pond, and Sucker Lake had abnormal late winter steroid profiles, in that testosterone production had not increased or had decreased as compared with fall values. Yellow perch from the other three sites showed the increase in testosterone that would be expected based on the rainbow trout model. It is unlikely that the responsible mechanism is consistent among the three affected sites, since Sucker Lake yellow perch did not have similar levels of exposure to oil sands compounds as compared with yellow perch from the two experimental ponds. This suggests that these observations may not have been directly mediated by any chemicals present. The mechanism of response in Sucker Lake may be one of general stress in light of the low levels of dissolved oxygen during the winter ($2.0\ mg \cdot L^{-1}$, van den Heuvel et al. 1999). Elevated stress levels have been observed to have a potent effect on steroid synthesis (Jardine et al. 1996). The possibility that the alterations in late winter steroid profiles seen in Demonstration Pond and South Bison Pond yellow perch were directly or indirectly caused by contaminants remains. However, dietary and abiotic factors leading to stress, or indirect contaminant stress, in these organisms cannot be ruled out.

Another key finding was that the alterations in yellow perch steroid hormone profiles could not be related to gonad size indices in individuals. Differences in steroid levels among sites observed here varied by as much as 10-fold, yet were not correlated with gonad size. This raises questions relating to the use of steroids as monitoring tools. The magnitude of steroid change required to cause an alteration in gonadal growth remains essentially unknown. As with any toxicological response, the levels of steroid change required to cause a particular gonadal effect are probably highly dependent on modifying factors, particularly nutrition. Evidence from this study (van den Heuvel et al. 1999) indicates that South Bison Pond yellow perch in all probability had relatively high energy intake and storage as compared with yellow perch from many of the other sites. This improved nutritional regime may have been sufficient to mask physiological effects mediated by depressed steroid levels. Steroids were also measured at particular points in time during vitellogenesis. It must be recognized that gonad growth occurs over many months and steroids in the winter period (when the most significant differences occurred) may not be relevant to gonad growth that has occurred previously during the year.

This study has identified biochemical and chemical indicators of exposure to oil sands related compounds. However, due to the lack of relationship between these parameters and observations at the individual or physiological level, the measured indicators remain indicators of exposure, not indicators of toxicological effect. Our study could not factor out the effect of many biotic and abiotic modifying factors that invariably influence field-based measures. This raises obvious questions about the utility of the "biomarker" or "bioindicator" approach for making inferences about effects likely to be observed at higher levels of biological organiza-

tion. However, these observations do not detract from the value of biochemical tools when used to gain insight into the mechanisms of impaired physiological performance.

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