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The effect of pulp and paper mill effluent on an insectivorous bird, the tree swallow

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Pulp mill effluent is known to affect freshwater biota in various ways. However, its effects on riparian birds that feed on insects emerging from aquatic ecosystems have not been examined. This study examined diet, circulating sex steroids, highly carboxylated porphyrins (HCPs), activity of the mixed function oxygenase enzyme 7-ethoxyresorufin-*O*-deethylase (EROD), the liver somatic index and reproductive performance and nestling size in tree swallows, an insectivorous bird, at sites located upstream and downstream from two pulp mills in western Canada during 1993–1996. The tree swallow diet consisted of 50–60% insects of aquatic origin. In general, physiological biomarkers in tree swallows located downstream from the pulp mill effluents did not differ from those at upstream sites, suggesting that dietary exposures to pulp mill effluents at these sites were insufficient to elicit responses. Nevertheless, it is noteworthy that 17β-oestradiol was lower in incubating females at a site downstream from one of the pulp mills in 1 of 2 years. In addition, HCPs in tree swallows downstream from the other pulp mill were elevated significantly. Reproductive performance by tree swallows did not differ significantly between upstream and downstream locations at either mill although there was a definite trend towards enhanced reproduction at downstream sites. At both pulp mills, 16 day old nestlings were significantly larger at downstream sites compared to their counterparts at upstream sites in at least 1 year. The improved reproduction and larger nestlings at downstream sites may be the result of a greater food supply, consistent with the nutrient enrichment effect often seen below pulp mills.

Keywords: pulp mills, tree swallows; riparian wildlife; biomarkers.

Introduction

Pulp and paper mill effluent contains nutrients and contaminants (Owens, 1991) and has the capacity to alter the receiving environment in several ways. For this reason, there has been much public concern in recent years over the potential effects of pulp mill effluents on the ecosystems they enter.

The toxic responses of aquatic organisms to pulp mill effluent, in particular to bleached kraft mill effluent (BKME), have been investigated widely (Government of Canada, 1991; Owens, 1991; Munkittrick *et al*., 1994). Fish exposed to effluents from some pulp mills have often displayed mixed function oxidase (MFO) induction (reviewed by Hodson, 1996) and altered levels of circulating sex steroids (McMaster *et al*., 1991, 1996; Munkittrick *et al*., 1991, 1992a,b, 1994; Gagnon *et al*., 1994). At some pulp mills, additional effects have been seen, including enlarged livers relative to body size, decreased growth rates, changes in body condition, decreased investment in reproduction and/or delays in sexual maturation (McMaster *et al*., 1991; Munkittrick *et al*., 1991, 1992a; Hodson *et al*., 1992; Gagnon *et al*., 1995). At one pulp mill, multivariate analyses suggested that fish living immediately downstream were in poorer health than a comparable population living upstream from the mill (Adams *et al*., 1996). The chemicals in pulp mill effluent that are responsible for these effects have not been identified with any certainty (Munkittrick *et al*., 1994; Hewitt *et al*., 1996; Hodson, 1996; Servos, 1996). While some of these effects are likely mediated by waterborne chemicals, food chain transfer of chemicals that cause

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these effects has not been eliminated as a possible route of exposure.

Nutrient enrichment of receiving environments is another phenomenon associated with some pulp and paper mills (Bothwell, 1992). Field studies have indicated that structural and functional impacts are evident in benthic invertebrates (Anderson, 1989; Haley *et al*., 1995; Cash *et al*., 1996; Lowell *et al*., 1996). The consequences of enrichment for organisms that are higher in the food chain have not been examined closely, except in the case of certain predatory fish (Munkittrick *et al*., 1991, 1992b; Hodson *et al*., 1992; Kloepper-Sams *et al*., 1994; Swanson *et al*., 1994).

There is widespread recognition that emergence of aquatic insects represents an important source of nutrients and contaminants of aquatic origin in terrestrial (usually riparian) ecosystems (Wrubleski, 1987; Kovats and Ciborowski, 1989). However, there has been no provision made for incorporating a riparian component into the environmental monitoring programmes that Canadian pulp mills are required to undertake as part of the Environmental Effects Monitoring (EEM) regulations implemented under the Canadian Fisheries Act (Environment Canada and Fisheries and Oceans Canada, 1992). Before recommending that riparian monitoring be included within the scope of pulp mill EEM programmes, it is necessary to determine whether there is any evidence of impacts of pulp mill effluent on riparian wildlife.

Tree swallows (*Tachycineta bicolor*) are abundant, widely distributed, aerial insectivores. In riparian areas, their diet consists of approximately 50–90% emerged aquatic insects (Quinney and Ankney, 1985; Blancher and McNicol, 1991). Tree swallows belong to an aerially insectivorous guild that is commonly associated with riparian habitats (Holroyd, 1983). They are cavity nesters that readily nest in boxes provided by humans and exhibit strong site tenacity and forage over short distances from their nests during the breeding season (St Louis *et al*., 1990; Robertson *et al*., 1992). The localized foraging range of tree swallows means that they will integrate food chain effects from a localized area and previous studies have indicated that tree swallows can be effective monitors of local pollution (Ankley *et al*., 1993; St Louis *et al*., 1993; Bishop *et al*., 1995) and that they may be affected by human-induced alterations to aquatic ecosystems (Blancher and McNicol, 1988). Their reproductive effort and success and the growth and survival of their offspring is sensitive to food availability (Quinney *et al*., 1986; Dunn and Hannon, 1992). Thus, pulp mill effluent-induced changes in the availability of emerged insects could have consequences for tree swallow reproduction.

In this study, we examined selected physiological and reproductive parameters in tree swallows breeding upstream and downstream from two modern bleached kraft pulp mills in western Canada in order to determine whether the effects of exposure to pulp mill effluents that previously have been observed in fish are similar in this riparian bird species.

Study area

Our study was conducted at sites located upstream and downstream from two bleached kraft pulp mills in western Canada (Fig. 1). One of the mills, located near Grande Prairie, Alberta, produces approximately 800–850 t of air-dried pulp per day. Its feedstock consists of 70% spruce (*Picea*) and 30% pine (*Pinus*). The bleaching process is as follows: $D_0E_{op}D_1ED_2$ where D_0 refers to chlorine dioxide, E_{op} to caustic extraction with oxygen and peroxide, D_1 to chlorine dioxide, E to caustic extraction and D_2 to chlorine dioxide. Effluent treatment consists of several stages, the most significant of which are as follows: (1) primary treatment in a clarifier where significant quantities of solids are removed, (2) 17 h retention in a settling basin to allow material to settle and (3) secondary (biological) treatment in an aerated stabilization basin with a retention period of 15 days. Effluent flow to the Wapiti River is 50 000–60 000 m^3

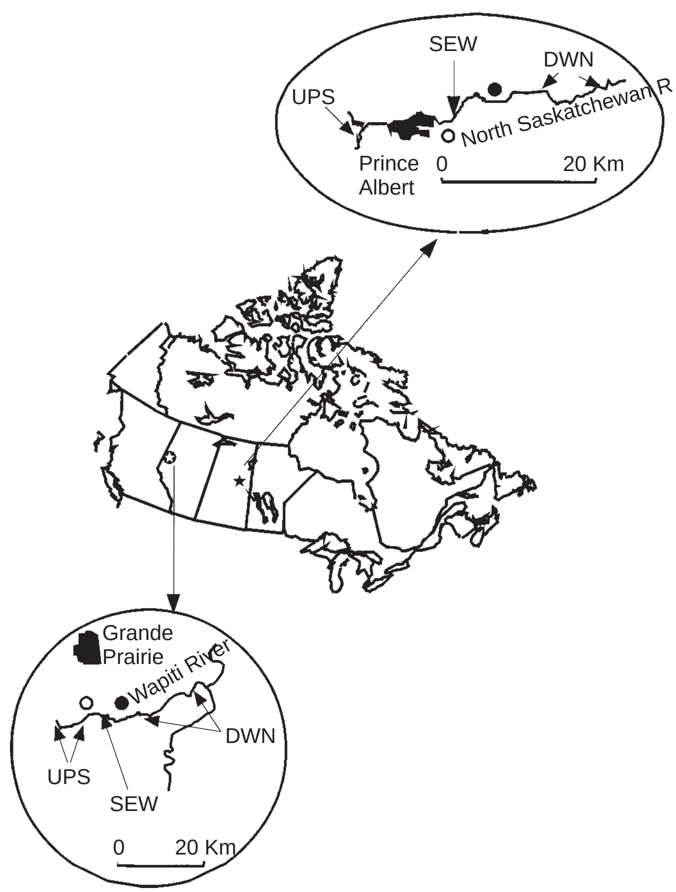

Fig. 1. Location of pulp mills and of study sites relative to pulp mill and municipal effluents. UPS, upstream; SEW, sewage; DWN, downstream; ✪, Grande Prairie; ★, Prince Albert; ○, sewage treatment; ●, pulp mill.

day^{-1}. The mean annual flow rate in the Wapiti River based on 32 years through to 1991 was 9×10^6 m^3 day^{-1}. Thus, the effluent accounts for a mean of 0.6% of the total river flow over the year. However, because of the large seasonal variation in river flow, effluent may account for up to 12% of river flow at certain times of the year (Swanson *et al*., 1993). The effluent characteristics are shown in Table 1.

The second mill, located on the North Saskatchewan River near Prince Albert, Saskatchewan, uses both hardwoods (aspen) and softwoods (jackpine and spruce) as feedstock. There are six stages to the bleaching process: (1) chlorine dioxide : chlorine (80% ClO_2 : 20%Cl), (2) caustic wash, (3) caustic extraction with NaOH and O_2, (4) first ClO_2, (5) second caustic extraction (NaOH) and (6) second ClO_2. Effluent passes into primary settling basins. Clarified effluent then enters an aerated secondary (biological) treatment lagoon with an 8 day retention period. Final effluent enters the North Saskatchewan River at a rate of approximately 100 000 m^3 day^{-1}. During 1993 and 1995, the years when this study was conducted at Prince Albert, the daily river flow averaged over the 2 years was 19×10^6 m^3 day^{-1}. Thus, effluent accounted for approximately 0.5% of the river's volume averaged over the year. However, during periods of low flow, effluent may account for up to 1% of the volume. The effluent characteristics are shown in Table 1.

Methods

Experimental design

At Grande Prairie, nest boxes were placed near the river's edge along threee reaches of the river.

(1) An upstream or reference reach situated 16–20 km upstream of the pulp mill effluent diffuser and 2.5–6.5 km upstream from the sewage outfall for the city of Grande Prairie.
(2) A second reach located 12.0–12.5 km upstream from the mill's effluent diffuser and 1.0–1.5 km downstream from the city sewage outfall (sewage site).
(3) A downstream or exposed reach located 3–25 km downstream from the mill (Fig. 1).

Breeding pairs of tree swallows were allowed to colonize the boxes and various data were collected throughout the breeding season (see below).

At Prince Albert, nest boxes were placed near the river's edge at four sites

(1) An upstream or reference site located approximately 25 km above the pulp mill diffuser and 12 km above the Prince Albert city sewage outflow.
(2) A site located approximately 1 km below the city sewage outflow (sewage site).
(3) A near-downstream or exposed site approximately 7 km below the pulp mill diffuser.
(4) A far-downstream site approximately 18 km below the pulp mill diffuser (Fig. 1).

Table 1. Characteristics of final effluent at the Grande Prairie and Prince Albert pulp mills[a]

Parameter	Grande Prairie	Prince Albert
Effluent flow (m^3 day^{-1})	55 000.00	107 000.00
AOX (mg l^{-1})[b]	7.80	12.50
NO_2^- (mg l^{-1})	0.24	0.07
NO_3^- (mg l^{-1})	0.37	0.15
TKN (mg l^{-1})[c]	–	6.50
DOC (mg l^{-1})	–	118.00
Total suspended solids (mg l^{-1})	20.00	34.00
Total P (mg l^{-1})	1.00	0.90
BOD (mg l^{-1})	23.00	23.00
Sulphides (mg l^{-1})	0.10	–
SO_4^- (mg l^{-1})	–	192.00
Total fatty acids (μg l^{-1})	–	91.00
Total resin acids (μg l^{-1})	–	69.00

[a]Values based on unpublished data graciously supplied by each of the pulp mills.
[b]AUX – adsorbable organic halides.
[c]TKN – total kjeldhal nitrogen.

Examination of tree swallow diet

In order to determine whether tree swallows were feeding on insects of riverine origin, we collected food samples fed to the nestlings by the parents at both Grande Prairie (1994–1995) and Prince Albert (1993 and 1995). Ligatures made from polyethylene-covered copper wire were placed around the throats of 8–12 day old nestlings for up to 30 min in order to prevent them from swallowing food (Johnson *et al*., 1980). Food items were then removed from the birds and placed temporarily in 70% ethanol. Within 4 h, the ethanol was drained from the sample, insects were thoroughly rinsed in water, allowed to dry partially, placed in glass vials and frozen. Insects obtained from all nestlings in a nest at a given sampling time constituted a sample. Insects were counted, identified to family (Diptera only) or order (all other groups) and measured to the nearest 2 mm. Representative samples of each 2 mm size class for each taxon were oven dried to constant weight and weighed to determine the mean weight of a given insect in that taxon size class. Each representative sample usually consisted of 100 individuals. The numbers of each taxon size class were then converted to a biomass estimate using these mean weights. Then, each taxon was classified as (1) of lotic origin, (2) possibly of lotic origin, (3) of non-lotic origin or (4) of unknown origin, according to whether the particular taxon had larval forms that were likely to occur in the river. The group classified as possibly of lotic origin consisted of several families of dipterans that are comprised of terrestrial, lentic and lotic taxa which we were not able to differentiate. In reporting the relative importance of a particular group of insects in the diet of tree swallows, we have assigned each sample equal weight regardless of the actual number or biomass of insects it contained (Swanson *et al*., 1974).

Physiological biomarkers

Following examples from studies on the effects of pulp mill effluent on fish (McMaster *et al*., 1991; Munkittrick *et al*., 1991, 1992a, 1994; Kloepper-Sams *et al*., 1994; Xu *et al*., 1994; Haley *et al*., 1995), we examined several physiological end-points in tree swallows that, in fish, have been shown to be responsive to exposure to pulp mill effluent.

The activity levels of the MFO enzyme 7-ethoxyresorufin-*O*-deethylase (EROD), liver somatic indices and porphyrin levels were determined in 16 day old nestlings in 1993 at Prince Albert and in 1995 at Grande Prairie. Tree swallow nestlings usually fledge at 18–22 days old and, thus, the 16 day old nestlings were nearly full grown. At Prince Albert, one to three nestlings were randomly selected from each nest and, at Grande Prairie, one nestling was randomly selected from each nest for analysis. The bird was weighed, killed by cervical dislocation and its liver was removed, weighed and placed in liquid nitrogen within 10 min of death.

EROD and porphyrin analyses were done at the National Wildlife Research Centre, Hull, Québec, Canada. EROD activities were measured by modifications of published methods (Pohl and Fouts, 1980; Klotz *et al*., 1984). For EROD analysis, approximately 200 mg subsamples of minced liver were homogenized on ice in a Potter-Elvehjelm tube with a Teflon pestel in four volumes of 0.1 M phosphate buffer at pH 7.4. The liver homogenate was kept on ice during the entire procedure. Cell debris was removed by centrifuging for 15 min at 9000 *g*. Microsomes were prepared by gel filtration based on the method of Pyykko (1983) and aliquots were kept frozen in liquid nitrogen until EROD activity determination which was performed in 24 well plates. The reaction mixtures consisted of 355 μl (sample well) and 430 μl (blank well) of 0.1 M Tris buffer (pH 8.0) containing 0.1 M NaCl. 100–200 μg of microsomal protein, 50 μl of 0.1 M $MgCl_2$ and 20 μl of 50 μM 7-ethoxyresorufin prepared in (1 : 9) MeOH : 0.1 Tris buffer (pH 8.0) containing 0.1 M NaCl were added to each well. The final substrate concentration in the reaction mixture was 2 μM. The plate was pre-incubated at 37 °C for 5 min on an agitator. The reaction was started by adding 25 μl of 12 mM NADPH freshly prepared in buffer to the reaction wells (but not the blank wells). The reaction was allowed to proceed for 10 min after which 1 ml of chilled methanol was added to all wells. The plates were then scanned for resorufin using the Cytofluor 2300 Fluorescence Measurement System set up at 530 nm for the excitation wavelength (25 nm bandwidth) and 590 nm for the emission wavelength (35 nm bandwidth). The amount of resorufin formed in the reaction wells was calculated from a resorufin standard curve. Microsomal protein concentrations were determined by Lowry's method as simplified by Peterson (1977) using a bovine serum albumin (BSA) standard curve. The enzyme activity is represented as a mean of three replicates and is expressed as pmol min^{-1} per mg microsomal protein. The lower limit of detection for EROD activity was 3 pmol min^{-1} per mg protein. A quality assurance sample consisting of an aliquot of a homogenous microsomal preparation was included on each plate. EROD analyses were performed in duplicate or triplicate and the precision averaged 3% and ranged from $\pm$1–10% of the mean.

Hepatic porphyrin concentrations were determined following the method of Kennedy and James (1993) with a few modifications. The analyses were carried out with a Varian LC Star System and a Perkin-Elmer LC-240 fluorescence detector equipped with a Hamatsu R-928 red-sensitive photomultiplier tube.

The liver somatic index (LSI) is the fresh liver weight adjusted to a standardized residual body weight (total body weight – liver weight) by using the residual body weight as a covariate in analyses.

For determination of sex steroids (androgen and 17β-oestradiol), blood samples were drawn from female tree swallows that had been incubating eggs for 6–12 days. In this way, sex steroids were assessed at a reasonably standardized stage of their reproductive cycle. Samples were centrifuged and plasma was drawn off into micro-centrifuge tubes and frozen. In the laboratory, samples were analysed using a radioimmunoassay (RIA) technique according to Van Der Kraak *et al*. (1984). To eliminate possible interference from plasma steroid-binding proteins, steroid measurements were done on reconstituted organic diethyl ether extracts of the plasma samples. The plasma sex steroid extraction efficiency was estimated to be consistently greater than 90%. Each extract was measured in duplicate at two dilutions (1 : 2 and 1 : 4 v/v relative to the original plasma sample). The inter- and intra-assay coefficients of variation were less than 5%. Antisera for the analysis were obtained from Sigma Chemicals. The testosterone antiserum cross-reacts with testosterone and 5α-dihydrotestosterone. 5α-Dihydrotestosterone is known to be biologically active in birds (Wingfield and Farner, 1980). Therefore, the RIA results for testosterone are best represented as androgen levels. Practical detection limits were 50 pg ml^{-1} for 17β-oestradiol and 200 pg ml^{-1} for androgens. Samples in which sex steroids were not detected were assigned values of 0.5 times the detection limit.

Reproduction and nestling growth

Reproduction and nestling size at 16 days of age were monitored at Prince Albert in 1993 and 1995 and at Grande Prairie in 1994–1996. For each nest box the following data were collected: age class of the nesting female (second year (SY) or after second year (ASY)) according to plumage characteristics (Hussell, 1983), clutch size, clutch mass, clutch initiation date, hatch date, number of eggs that hatched, survival of nestlings to 16 days old and the following measurements of nestling size at 16 days old – weight, tarsal length, ninth primary feather length and wing length. The body size measurements were used to develop a single overall measurement of body size using principal components analysis (Dillon and Goldstein, 1984).

Insect abundance/biomass

At Prince Albert, the abundance and biomass of flying insects was estimated in 1993 and 1995. Flying insects were collected in two stationary aerial tow nets at each site following the example in Quinney and Ankney (1985). Aerial tow nets are passive samplers that collect wind-driven flying insects. We collected samples for approximately 20–28 h each sampling period, 48 times in 1993 and 23 times in 1995. The collections began at the beginning of egg laying and finished when nearly all of the nestlings had fledged. In 1993, samples were collected nearly every day while in 1995 they were collected 4 days each week. The elapsed time between opening and closing the traps each day was recorded. Wind speed was also recorded at each trap nearly every day. Hourly wind speeds at each net were estimated by regression equations based on wind speeds recorded at the net and hourly wind speeds recorded at the Environment Canada Prince Albert weather station. A wind speed index for each trap was then calculated by summing the estimated hourly wind speeds each day according to a method described in detail by Quinney *et al*. (1986). Samples were stored in 70% ethanol in the field. Later they were sorted to order or family (dipterans only), size classed in 2 mm intervals and counted. Counts were adjusted to a standardized (24 h) period and a constant wind speed according to Quinney *et al*. (1986). For each taxon size class, biomass estimates were determined as described above. Abundance values were converted to biomass by multiplying each abundance score by the estimated biomass for that taxon size class. Insects were then classified as lotic, possibly lotic, non-lotic or unknown according to their larval habitat as described above. For each sample day, estimates of biomass of each of these groups of insects were determined at each site by averaging values from the two traps. Then, for each sampling day, values were ranked from lowest to highest. Using this approach, it was possible to estimate consistently whether the sites differed from each other on a day-to-day basis.

Statistics

In all analyses of the tree swallows, the nest was used as the experimental unit. When we had measurements on several nestlings within a nest, mean values were used. Data from Grande Prairie and Prince Albert were analysed separately. Raw or log-transformed data were checked for homogeneity of variances using the F_{max} test (Sokal and Rohlf, 1981) and for normality using the Shapiro–Wilk statistic (SAS Institute, 1988). For data sets satisfying these conditions, analysis of variance (ANOVA) was used to test for between-site differences. When differences were found, *a posteriori* multiple comparisons were made using the Tukey–Kramer procedure. Where appropriate, analyses were performed initially using full models, that is the effects of year and female age were included. If either of these variables was not significant at the $\alpha = 0.10$ level it was removed and the analysis was repeated on the reduced model. We initially included female age as a possible factor influencing the response variables because other studies have shown that reproductive outcomes differ between SY and ASY females (Desteven, 1978; Blancher and McNicol, 1988; Stutchbury and Robertson, 1988). Following the example of Blancher and McNicol (1988), when female age had a significant effect on a given response variable, only nests of SY females were included in the analysis. We arbitrarily selected SY females because there were more of them in this study than ASY females. In addition, for analyses of clutch mass, nestling survival and nestling size, we initially examined the relationship between clutch initiation date and these variables using linear correlation because tree swallows that commence egg laying early in the year tend to have larger clutches and more young than those that start laying later (Blancher and McNicol, 1988; Stutchbury and Robertson, 1988). When the clutch initiation date was significantly correlated ($p < 0.1$) with one of these variables it was included as a covariate when examining the effects of site. When variances were not homogeneous or data was not normal, non-parametric Kruskal–Wallis tests or ANOVAs on ranked data (Conover and Iman, 1981) were used to test for between-site differences.

For analysis of between-site differences in insect biomass at Prince Albert, a repeated measures ANOVA was used on ranked data. Probabilities were calculated using a randomization procedure with 1000 iterations. This procedure was applied to relieve the need for meeting assumptions of parametric tests (Edgington, 1995), assumptions which the insect data did not meet. When significant differences were found, multiple comparisons tests were performed (Edgington, 1995).

Results

Diet

At Grande Prairie, 60 dietary samples containing 3522 insects were collected. Lotic insects, primarily heptageniid mayflies, accounted for 53% of the weighted total biomass (Fig. 2). Non-lotic insects accounted for 32% of the weighted total biomass and insects that were possibly lotic accounted for 15% of the weighted biomass. At Prince Albert, 32 dietary samples containing 702 insects were collected. Lotic insects, primarily hydropsychid caddisflies and heptageniid mayflies, accounted for 57% of the

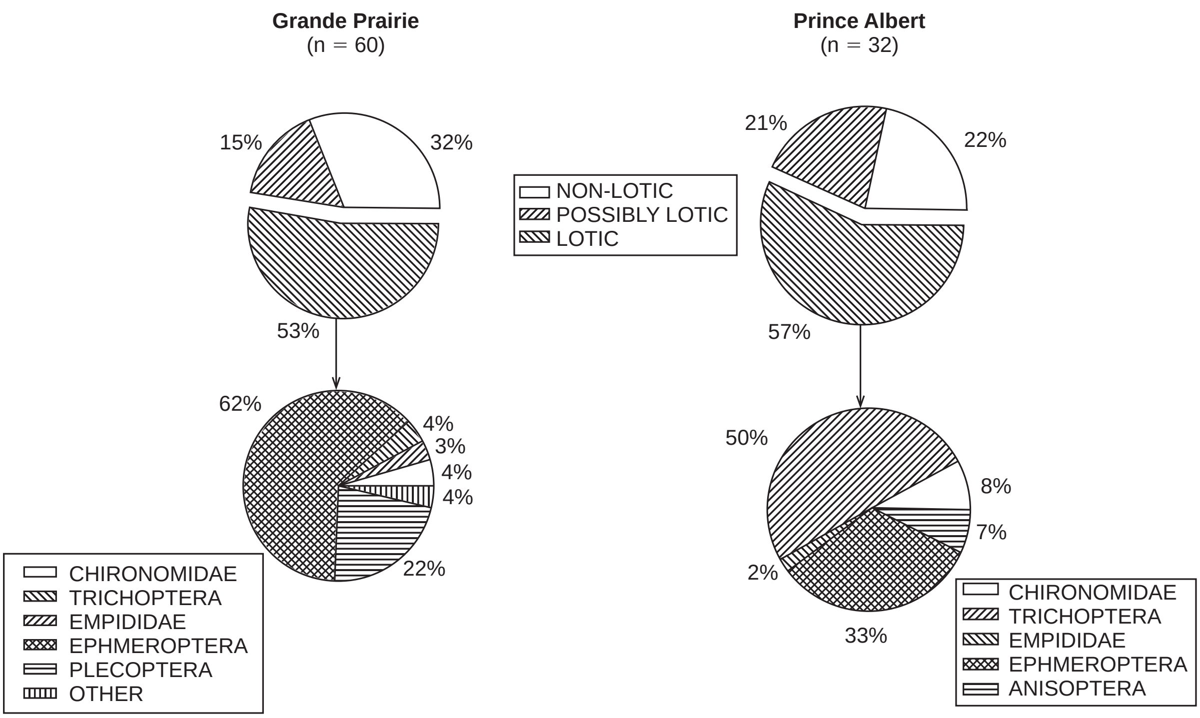

Fig. 2. Diets of tree swallow nestlings. Top charts show the relative proportions of insect groups in the total diet according to the habitats used by immature forms. Bottom charts show the relative proportions of the different lotic taxa in the lotic portion of their diet.

weighted total biomass, while non-lotic insects accounted for 22% and possibly lotic insects for 21% of the biomass (Fig. 2).

Physiological biomarkers

Only 17β-oestradiol and HCPs, defined as the sum of uroporphyrin, heptaporphyrin, hexaporphyrin and pentaporphyrin (see Fox *et al*., 1988; Xu *et al*., 1994), showed evidence of change from upstream to downstream in a manner consistent with exposure to pulp mill effluent. However, for HCPs this was evident at the Prince Albert mill only. At that study area, livers of 16 day old nestlings from the downstream site closest to the pulp mill had HCP levels that were significantly higher than those from the sewage and downstream site furthest from the mill (ANOVA, $p = 0.02$ and *a posteriori* Tukey–Kramer, $p < 0.05$). The mean hepatic HCP level in tree swallows from that site averaged 1.4–1.6 times higher than those from other sites (Table 2). When protoporphyrin was used as a covariate in the analysis, the result was similar: at Prince Albert, HCPs were significantly higher at the downstream site closest to the mill when compared to other sites (ANCOVA, $p = 0.02$ and *a posteriori* multiple comparison of near-downstream site to all other sites, $p < 0.1$). Mean HCP adjusted to a common protoporphyrin value was in the range of 1.4–1.9 times higher at that site when compared to other sites (Table 2).

17β-oestradiol was lower in tree swallows potentially exposed to pulp mill effluent only at the Grande Prairie study area and only in 1 of the 2 years in which sex steroids were examined at that study area. At Grande Prairie, concentrations of 17β-oestradiol in plasma of incubating female tree swallows differed between sites in a pattern that was not consistent between years (ANOVA, year $\times$ site interaction, $p = 0.0004$). Therefore, we examined the effects of site for each year. In 1995, 17β-oestradiol was significantly lower in tree swallows from the downstream reach compared to the upstream reach (ANOVA, $p = 0.005$ and Tukey-Kramer, $p < 0.1$, comparing downstream site to upstream and sewage sites). The mean concentration of this hormone in swallows from the downstream reach was only approximately 0.6 times as high than in those from the other reaches of the river (Table 3). However, this pattern was not repeated in 1996. In that year, 17β-oestradiol in plasma of incubating tree swallows on the upstream reach of the river was relatively low, particularly when compared to their counterparts from the downstream reach (ANOVA, $p = 0.02$ and Tukey-Kramer, $p < 0.05$, comparing upstream and downstream reaches). The mean concentration of 17β-oestradiol in birds from the downstream reach was three times higher than in birds from the upstream reach in 1996 (Table 3).

Other biomarker responses that were measured, including EROD, LSI and androgens, did not differ between sites in a manner that was consistent with a possible pulp mill effluent effect. However, some significant differences were

Table 2. Mean ± SE or median (quartiles) values and sample sizes for the indicated variables in tree swallows at study sites upstream and downstream from the Prince Albert pulp mill

Variable	Year	Upstream	Sewage	Downstream-near	Downstream-far
EROD (pmol min^{-1} per mg protein)[a]	1993	8.8A (6.4–10.9) 6	14.3A (10.5–23.6) 10	9.6A (6.7–11.5) 8	12.6A (8.0–19.5) 8
HCPs (pmol per g liver)[b]	1993	48AB (39–58) 6	42A (34–51) 8	68B (51–92) 4	42A (31–57) 5
HCPs : proto (pmol per g liver)[c]	1993	50A (37–67) 6	46A (36–58) 8	71B (45–112) 4	38A (26–54) 5
LSI (g)[c]	1993	1.2A (1.0–1.5) 6	1.2A (1.1–1.3) 12	1.1AB (0.9–1.2) 8	0.9B (0.8–1.2) 8
17β-oestradiol (pg per ml plasma)[d]	1995	208 ± 19A 6	177 ± 11A 13	206 ± 10A 13	203 ± 12A 8
Androgens (pg per ml plasma)[a]	1995	213A (100–219) 10	302B (232–454) 12	154AB (100–259) 12	272AB (100–536) 6
Clutch mass (g)[e]	1993	9.9 ± 0.6A 6	10.3 ± 0.5A 11	11.0 ± 0.6A 6	11.1 ± 0.6A 7
	1995	9.4 ± 0.5A 9	10.3 ± 0.6A 6	10.1 ± 0.5A 8	9.3 ± 0.7 5
Survival in hatched nests[f]	1993 and 1995	3.8 ± 0.5A 19	4.4 ± 0.4A 24	4.6 ± 0.4A 25	5.2 ± 0.2A 16
Survival in successful nests[f]	1993 and 1995	4.8 ± 0.3A 15	4.9 ± 0.3A 22	5.2 ± 0.3A 22	5.1 ± 0.3A 16
Nestling size[g]	1993	−2.4 ± 0.4A 5	−1.1 ± 0.3B 12	−0.6 ± 0.4B 7	0.3 ± 0.3C 8
	1995	0.4 ± 0.3A 10	0.9 ± 0.3A 10	0.5 ± 0.2A 15	0.7 ± 0.3A 8

[a]Median (lower–upper quartiles) in nestlings for EROD and nesting females for androgens.
[b]Mean (95% CLs) backtransformed from $\log_e$ values in 16 day old nestlings.
[c]Least-squared mean (95% CLs) backtransformed from $\log_e$ values, adjusted value of covariate.
[d]Mean ± SE in nesting females.
[e]Least-squared mean ± SE for clutches of SY females adjusted to a common clutch initiation date.
[f]Least-squared mean ± SE for nests of both female ages adjusted to a common clutch initiation date. Years are combined because neither year nor year × site were significantly related to survival.
[g]Mean ± SE first principal component estimate of 16 day old nestling size in nests of females of both ages.
Within a row, values followed by similar upper case letters are not significantly different ($p > 0.05$).

noted. At Prince Albert, androgen concentrations in incubating females at the sewage site were significantly greater than those in tree swallows from the upstream site ($p = 0.03$; Table 2). In addition, at Prince Albert, the LSI in 16 day old nestlings was significantly lower in birds from the far-downstream site compared to the upstream and sewage sites ($p \leqslant 0.01$; Table 2), a pattern that is opposite to what has been observed in fish downstream from pulp mills (Munkittrick *et al*., 1991; Hodson *et al*., 1992; Kloepper-Sams *et al*., 1994). At Grande Prairie, nestlings on the downstream reach had a lower mean LSI than their counterparts on the upstream reach ($p < 0.05$) but a higher mean LSI than those at the sewage site ($p < 0.05$; Table 3). Hepatic EROD activity in 16 day old nestlings did not differ between sites at either study area (Kruskal–Wallis, $p > 0.1$). HCPs did not differ between reaches at Grande Prairie ($p = 0.4$). At Grande Prairie, HCPs in nestlings and androgens in incubating females did not differ between sites (ANOVA on ranked data, $p > 0.4$).

Reproduction and nestling body size

Female age class had a significant effect on (1) clutch mass at both study areas (Grande Prairie, $p = 0.008$ and Prince Albert, $p = 0.004$; Table 4) and (2) our measure of 16 day old nestling size at Grande Prairie ($p = 0.05$; Table 4). Therefore, in subsequent analyses of clutch mass and nestling size at Grande Prairie, only nests of SY females are considered.

The clutch initiation date was significantly correlated ($p < 0.05$) with clutch mass and nestling survival at both study areas and with the first principal component measure of body size (see below) at Grande Prairie (Table 5). Therefore, in subsequent analyses pertaining to these variables, clutch initiation date is included as a covariate.

At both study areas, there were no significant differences between sites in clutch mass, survival of nestlings in nests where at least one egg hatched and survival of nestlings in nests that were successful in fledging at least one young ($p > 0.2$ for all analyses). However, there was a trend towards greater clutch mass and improved nestling survival downstream of the pulp mills (Tables 2 and 3).

Principal components analysis was used to develop a single variable that accounted for much of the variation in body size of 16 day old nestlings based on the four measurements that were taken. At Grande Prairie and Prince Albert, the first principal component accounted for 69 and 60%, respectively, of the variation in body size (Table 6). At both study areas, our four measurements of body size loaded positively on the first principal component indicating that high PC1 scores represent large nestlings while small PC1 scores represent small nestlings.

At both study areas, the relationship between site and PC1 scores differed within or between years (Grande Prairie ANOVA, year × site, $p = 0.07$ and Prince Albert ANCOVA, year × site, $p = 0.03$). Therefore, we examined

Table 3. Mean ± SE or median (quartiles) values and sample sizes for the indicated variables in tree swallows at study sites upstream and downstream from the Grande Prairie pulp mill

Variable	Year	Upstream	Sewage	Downstream
EROD (pmol min^{-1} per mg protein)[a]	1995	24A (17–31) 6	22A (15–29) 10	17A (9–29) 11
HCPs (pmol per g liver)[b]	1995	44A (30–63) 6	35A (28–43) 10	43A (31–59) 10
HCPs : proto (pmol per g liver)[c]	1995	43A (29–62) 6	37A (28–49) 10	41A (32–54) 10
LSI (g)[c]	1995	0.93A (0.80–1.08) 6	0.72B (0.65–0.80) 10	0.81A (0.74–0.89) 11
17β-oestradiol (pg per ml plasma)[d]	1995	159 ± 21AB 6	172 ± 15A 12	103 ± 12B 11
	1996	49 ± 24A 4	119 ± 13AB 11	143 ± 18B 12
Androgens (pg per ml plasma)[a]	1995–1996	217A (100–228) 10	228A (100–330) 23	217A (100–266) 22
Clutch mass (g)[e]	1994–1996	9.1 ± 0.53A 9	9.7 ± 0.4A 14	9.5 ± 0.3A 21
Nestling survival in hatched nests[f]	1994–1996	3.8 ± 0.5A 13	3.8 ± 0.3A 33	4.1 ± 0.4A 27
Nestling survival in successful nests[f]	1994–1996	4.0 ± 0.4A 12	4.8 ± 0.3A 28	4.7 ± 0.3A 23
Nestling size[g]	1994	0.5 1	−1.1 ± 0.7 5	1.0 ± 0.1 2
	1995	−0.8 ± 0.2A 6	−0.1 ± 0.5AB 2	0.1 ± 0.2B 7
	1996	−0.1 ± 0.3A 2	0.4 ± 0.2A 3	−0.3 ± 0.5A 5

[a]Median (lower–upper quartile) in nestlings for EROD and nesting females for androgens.
[b]Mean (95% CLs) backtransformed from $\log_e$ values in 16 day old nestlings.
[c]Least-squared mean (95% CLs) backtransformed from $\log_e$ values, adjusted to constant value of covariate.
[d]Mean ± SE in nesting females.
[e]SY females only, least-squared mean ± SE adjusted for clutch initiation date.
[f]All nests, least-squared mean number of 16 day old nestlings ± SE adjusted for clutch initiation date.
[g]Mean ± SE first principal component estimate of 16 day old nestling size in nests of SY females.
The effect of year was not included in analyses of androgens, clutch mass and both estimates of nestling survival since year and year × site were not significant ($p > 0.1$) in these analyses. Within a row, values followed by similar upper case letters are not significantly different ($p > 0.05$).

Table 4. Relationship between female age category and various response variables at Grande Prairie and Prince Albert (mean ± SE or median (quartiles))

	Grande Prairie		Prince Albert	
Variable	SY	ASY	SY	ASY
17β-oestradiol (pg ml^{-1})	127 ± 10	138 ± 12	191 ± 12	200 ± 8
Androgens (pg ml^{-1})	216 (100–252)	250 (155–327)	209 (100–224)	239 (100–349)
Clutch mass (g)	9.5 ± 0.3	10.6 ± 0.3	10.1 ± 0.2	11.3 ± 0.4
Nestling survival[a]	3.5 ± 0.4	4.4 ± 0.3	4.6 ± 0.3	4.3 ± 0.4
Nestling survival[b]	4.4 ± 0.2	4.8 ± 0.2	5.1 ± 0.2	4.9 ± 0.3
Nestling size[c]	−0.23 ± 0.17	0.28 ± 0.14	−0.45 ± 0.22	0.49 ± 0.13

[a]Number of 16 day old nestlings in nests where at least one egg hatched.
[b]Number of 16 day old nestlings in successful nests (at least one 16 day old nestling produced).
[c]First principal component of body size measurements.

Table 5. Pearson correlation coeffients, ($p > r$) between clutch initiation date and various measures of reproductive effort and success at Grande Prairie and Prince Albert

Variable	Grande Prairie	Prince Albert
Clutch mass[a]	−0.61 (0.0001)	−0.53 (0.0001)
Nestling survival in nests that hatched	−0.57 (0.0001)	−0.36 (0.008)
Nestling survival in successful nests	−0.29 (0.01)	−0.57 (0.0001)
16 day old nestling size (PC1 measure)	0.15 (0.38)[a]	−0.22 (0.06)

[a]Only nests of SY females are included since female age had an effect on these variables.

the relationship between site and PC1 scores separately for each year. At Grande Prairie, only during 1995 was there a significant relationship between site and PC1 (ANOVA, $p = 0.05$). The mean PC1 score for swallow nestlings at the downstream site was significantly greater than that of their counterparts at the upstream site (Tukey–Kramer, $p < 0.1$) and was also greater than that of nestlings at the sewage site, although not significantly (Table 3). In 1994

Table 6. Component loadings for 16 day old nestling measurements on first principal component (PC1) and % variance explained by PC1

Measured variable	Grande Prairie	Prince Albert
Weight	0.45	0.36
Tarsal length	0.42	0.42
Wing length	0.57	0.60
Ninth primary length	0.54	0.58
% variance explained	69.00	60.00

and 1996, there were no significant differences in PC1 scores between sites. In both of those years, the sample sizes were probably too small for meaningful comparisons (Table 3). At Prince Albert, the mean PC1 values differed significantly between sites in 1993 but not in 1995. In 1993, the mean PC1 score of nestlings was lower at the upstream site than at the other sites ($p < 0.05$) and it was higher at the far-downstream site than at the other sites ($p < 0.05$). Nestling size tended to increase progressively from upstream to downstream at Prince Albert (Table 2).

Insect biomass availability

Lotic insects accounted for 88% of the total biomass collected in stationary aerial tow nets in 1993 and 68% of the total biomass in 1995. For lotic and total insect biomass, there were significant differences between sites in both years ($p < 0.001$). In 1993, the rank orders of lotic and total insect biomass at the far-downstream and sewage sites were greater than those at the upstream and near-downstream sites ($p < 0.01$). In 1995, there was a greater biomass of total and lotic insects at the far-downstream site than at the other sites ($p < 0.01$). Comparisons of other sites did not yield significant differences ($p > 0.20$).

Discussion

Insects with immature lotic forms accounted for 50–60% of the diet of tree swallow nestlings at Grande Prairie and Prince Albert. Diets of nestling and adult tree swallows are very similar (Blancher and McNicol, 1991). Thus, it is highly likely that lotic insects were important for breeding adults as well. In spite of the potential for dietary exposure of tree swallows to pulp mill effluent components in this study, there was little evidence that they were being affected in a manner similar to what has been documented in fish downstream from many pulp mills. There are a number of possible explanations for the lack of effects in this study. First, the Grande Prairie and Prince Albert pulp mills may produce effluents that, when diluted in river water, are incapable of eliciting effects of the types commonly observed in biota downstream from pulp mills, irrespective of whether the exposure is dietary or waterborne. There is some evidence to support this. Both mills use relatively modern pulping and bleaching processes and effluent treatment techniques (see Study area description above) which are known to reduce effluent concentrations of chlorinated organics, resin acids, fatty acids, plant sterols and other substances associated with physiological and ecological impairments in exposed populations (Hewitt *et al*., 1996; Servos, 1996). Detailed studies of mountain whitefish (*Prosopium williamsoni*) at the Grande Prairie mill in 1991 indicated that MFO induction was strongly related to exposure to persistent compounds, probably chlorinated dioxins and furans (Kloepper-Sams and Benton, 1994). After dioxins and furans were nearly eliminated in mill effluent as a result of changes in pulp processing, the MFO-inducing capacity of the effluent declined (Kloepper-Sams *et al*., 1994). Although this contrasts with other studies that have found pulp processing and effluent treatment techniques are unrelated to the capacity of effluent to induce MFOs (Hodson, 1996; Martel *et al*., 1996), it suggests that at the Grande Prairie mill dioxins and furans alone were responsible for hepatic MFO induction in fish. At Grande Prairie, EROD activity in tree swallows was examined in 1995 by which time dioxins and furans had declined to relatively low levels in the Wapiti River ecosystem (Swanson *et al*., 1996a). At the Prince Albert mill, fish caught downstream from the mill in 1992 did not display elevated EROD activities compared to fish from a reference site (Swanson *et al*., 1996b), although it was never ascertained whether the ‘exposed’ fish were truly exposed to the effluent or whether they were transients. The absence of EROD induction in that study agrees with the results of this study. It is possible that, at the time of our study, the Prince Albert and Grande Prairie effluents, after dilution in the North Saskatchewan and Wapiti Rivers, respectively, were incapable of inducing MFO activity in biota exposed to the effluent either through waterborne exposures or via the food chain. At Grande Prairie, earlier studies on fish failed to find a clear pulp mill-related depression in sex steroids, although unexplained differences existed in the pattern and degree of change of 17β-oestradiol and testosterone between exposed and reference fish (Kloepper-Sams *et al*., 1994; Schryer *et al*., 1995). Furthermore, in studies on fish at the two mills, with one exception liver somatic indices did not differ between exposed and reference populations in a manner consistent with a pulp mill effect (Kloepper-Sams *et al*., 1994; Swanson *et al*., 1996b). This is similar to the results of this study wherein the liver somatic indices of tree swallow nestlings were not related to potential exposure to pulp mill effluent.

Another possible explanation for the lack of effects on tree swallows in this study is that chemicals in pulp mill effluent that cause adverse effects in fish may not be readily transferred to birds through food. Pulp mill effluent is a complex and poorly characterized chemical cocktail. It remains uncertain as to which chemicals are responsible for the effects that have often been documented in fish (Hewitt *et al*., 1996; Servos, 1996). Food chain transfer of contaminants is important only for chemicals with $\log K_{ow} > 4$ (Muir and Servos, 1996). Servos (1996) reported that the predicted $\log K_{ow}$ for an EROD-inducing pulp mill effluent extract was in the range of 2–4.5 while Hodson (1996) reported that MFO inducers in pulp mill effluent are predicted to have a $\log K_{ow}$ in the range of 4.5–5.1, ranges that unfortunately do not permit conclusions to be drawn about their potential for food chain transfer. Recently, Burnison *et al*. (1997) identified an MFO inducer in pulp mill effluent as a chlorinated stilbene. However, the potential for food chain transfer of this chemical was not stated. Plant sterols are important constituents of pulp mill effluent (Stromberg *et al*., 1996) and at least one of them, β-sitosterol, has been implicated in the depression of sex steroids observed in fish downstream from several pulp mills (Knutson *et al*., 1995). Short-term, waterborne exposures of fish to plant sterol mixtures have resulted in depression of sex steroids suggesting that bioconcentration from water is important in affecting sex steroid levels. However, the potential for food chain transfer of plant sterols is unknown. In addition, it remains uncertain whether plant sterols are the most important or only pulp mill chemicals responsible for the observed depression of circulating sex steroids in fish downstream from certain pulp mills. The reason for this uncertainty is that secondary treatment removes 50–90% of plant sterols from final effluent (Stromberg *et al*., 1996), yet studies have shown that it did not remove or even reduce the sex steroid-depressing capacity of certain effluents (Munkittrick *et al*., 1992b, 1994). If other chemicals in pulp mill effluent also have the capacity to depress sex steroids they remain unknown and, therefore, their capacity for food chain transfer remains unknown. Thus, it remains unclear whether dietary exposure or bioconcentration from water is the main exposure pathway resulting in these effects. If the latter is the main exposure pathway, then consumption of aquatic prey would not be a source of exposure for riparian species such as tree swallows.

Alternatively, tree swallows may not be a sufficiently sensitive indicator for some of the end-points we measured. In particular, cytochrome P-4501A activity, a biochemical end-point that has received much attention in fish as a biomarker of pulp mill effluent exposure (Kloepper-Sams and Benton, 1994; Hodson, 1996; Martel *et al*., 1996), has not been examined in great detail in tree swallows. Thus, its potential for induction by contaminants with an affinity for the Ah (aryl hydrocarbon) receptor, including unknown substances in certain pulp mill effluents, is poorly known in that species. In birds, there is wide interspecific variation in the sensitivity of cytochrome P-4501A to induction by contaminants with an affinity for the Ah receptor (Kennedy *et al*., 1996; Elliott *et al*., 1997; Lorenzen *et al*., 1997b); whether tree swallow cytochrome P-4501A has high potential for induction is not known. Nevertheless, it is noteworthy that MFO activity in tree swallow nestlings at a polychlorinated biphenyl (PCB)-contaminated site was elevated significantly compared to control birds, indicating that at least some cytochrome P-450s can be induced by certain xenobiotics in this species (Yorks *et al*., 1996).

Notwithstanding the apparent lack of physiological effects in this study, the possibility of some effects cannot be completely ignored. In 1 of the 2 years at Grande Prairie, 17β-oestradiol was lower in incubating female tree swallows along the downstream reach than in their counterparts upstream from the mill. This finding is consistent with those of a number of studies on fish at several pulp mills using an array of pulp processing and effluent treatment technologies (McMaster *et al*., 1991, 1996; Hodson *et al*., 1992; Munkittrick *et al*., 1992a,b, 1994; Gagnon *et al*., 1994). Van Der Kraak *et al*. (1992) showed experimentally that pulp mill effluent can disrupt both the secretion and metabolism of sex steroids at several sites on the pituitary–gonadal axis. While depressions in circulating levels of both oestradiol and testosterone have been commonly seen in female fish downstream from pulp mills, there is some evidence that oestradiol can be depressed in female fish below pulp mills without concomitant depressions in testosterone (Munkittrick *et al*., 1994; Brown *et al*., 1996), a phenomenon seen in this study as well. It should be noted that female tree swallows were sampled during incubation in this study, a period when levels of 17β-oestradiol and testosterone are low in birds (Bluhm, 1988; Wingfield and Farner, 1993). These hormones are functionally more important in the avian reproductive cycle and occur at higher concentrations during the pairing and pre-laying periods. Even if dietary exposure to pulp mill effluent can affect circulating sex steroid levels in incubating tree swallows as found in 1 of 2 years at one study area in this study, its capacity to affect these hormones during the more critical pairing and pre-laying periods remains unknown.

Hepatic HCPs were higher in 16 day old nestlings at the downstream site closest to the Prince Albert pulp mill than at other sites at that study area. This agrees with the higher HCP levels in lake whitefish (*Coregonus clupeaformis*) exposed to pulp mill effluent at Jackfish Bay, Ontario, reported by Xu *et al*. (1994). Those authors postulated that chemicals in the mill effluent interfered with the enzymes uroporphyrinogen decarboxylase and coproporphyrinogen oxidase resulting in an accumulation of HCPs. However, they did not identify the responsible chemicals. 2,3,7,8-TCDD and 2,3,7,8-TCDF were found at elevated levels in the Jackfish Bay ecosystem at the time of the study by Xu *et al*. (1994) (Servos *et al*., 1994); thus, they also may have been elevated in lake whitefish from Jackfish Bay. These contaminants and PCBs with dixin-like activity caused porphyrin accumulation, dominated by the HCPs uroporphyrin and heptacarboxylporphyrin, in avian embryo hepatocytes (Lorenzen *et al.*, 1997a) and in avian and mammalian liver tissue (Goldstein *et al.*, 1974; Miranda *et al.*, 1987). They also caused EROD induction (Kennedy *et al.*, 1996; Lorenzen *et al.*, 1997a) and it has been hypothesized that contaminants that cause both EROD induction and porphyrin accumulation do so via a common mechanism (Lorenzen *et al.*, 1997a). EROD induction was also seen in lake whitefish at Jackfish Bay in the study by Xu *et al.* (1994) but was not seen in the current study. Thus, it is possible that 2,3,7,8-TCDD and/or 2,3,7,8-TCDF may have been responsible for HCP accumulation in lake whitefish at Jackfish Bay. If so, the observed HCP accumulation in tree swallows downstream from the Prince Albert mill could not share a common cause with whitefish from Jackfish Bay because dioxins and furans are normally below detection limits in the Prince Albert pulp mill effluent and were below detection limits in fish collected downstream of the effluent input in the North Saskatchewan River (Swanson *et al.*, 1996b). Nevertheless, it is noteworthy that porphyrin accumulation can occur independently of EROD induction (Lorenzen *et al.*, 1997a) presumably via an unrelated mechanism. Thus, it remains possible that effluent from the Prince Albert mill may have contained a substance or substances that interfered with porphyrin metabolism in tree swallows. Additional research is needed to determine the sensitivity of porphyrin accumulation as a biomarker of animal exposure to effluents from various types of pulp mills.

While the physiological responses of tree swallows to dietary exposure to pulp mill effluents were either absent or uncertain, there appeared to be some effects on reproductive ecology and nestling growth at both study areas. For clutch size and nestling survival to 16 days old, these results were not significant. Nevertheless, in at least 1 year at each study area, a clear trend towards larger clutch masses and higher nestling productivity was evident at downstream sites when compared to upstream sites (Tables 2 and 3). Furthermore, 16 day old nestlings at downstream sites were larger than their counterparts at upstream sites in at least 1 year. These results may be attributable to a greater food supply in the form of emerged aquatic insects at the downstream sites. This was clearly demonstrated at the far-downstrem site at Prince Albert (Table 7) but not at the near-downstream site. The lack of agreement between the tree swallow reproductive and nestling growth data and the insect availability data at the near-downstream site may be explained by differences between their diets and the relative abundances of different insect taxa collected in the aerial tow nets. Together, Trichoptera and Ephemeroptera accounted for 83% of the lotic biomass in the dietary samples; however, they accounted for only 16 and 62% of the biomass of lotic insects collected in aerial tow nets in 1993 and 1995, respectively. In contrast, Chironomidae, which accounted for only 8% of the lotic insects in their diet, comprised 80 and 35% of the lotic insect biomass in aerial tow nets in 1993 and 1995, respectively. The stationary aerial tow nets appeared to be effective in sampling small, wind-driven insects such as chironomids but were less effective in sampling larger insects such as trichopterans and ephemeropterans.

Tree swallows exploit locally abundant food supplies (McCarty, 1997). Thus, they may take advantage of river reaches that have been enriched with nutrients by pulp mill or other effluents. Moreover, previous studies indicate that tree swallow reproductive performance and nestling growth are enhanced in areas of abundant food or, conversely, impaired in areas where food is not abundant. Quinney *et al.* (1986) found that tree swallow nestlings were larger at a food-enriched site compared to a site where there was less food available. Blancher and McNicol (1988) found that clutch sizes and volumes and nestling growth rates and sizes were lower in tree swallows on acidic wetlands when compared to those on non-acidic wetlands, differences that they attributed to an impoverished food supply on the acidic wetlands. Increased insect availability was associated with larger clutches (Hussell and Quinney, 1986; Dunn and Hannon, 1992) and higher fledging success (Dunn and Hannon, 1992) in tree swallows. Similarly, pollution-mediated abundance of invertebrates has been reported to affect reproduction and nestling growth in great tits (*Parus major*) (Eeva *et al.*, 1997). The trend towards better reproductive performance and the greater nestling growth in tree swallows at downstream sites compared to upstream sites is consistent with higher

Table 7. Mean rank order of total and lotic insect biomass collected in stationary aerial tow nets at Prince Albert in 1993 ($n = 48$) and 1995 ($n = 23$)

Variable	Year	Upstream	Sewage	Downstream near	Downstream far
Lotic insects	1993	1.6	3.2	1.9	3.3
	1995	2.2	1.8	2.2	3.8
Total insects	1993	1.6	3.2	3.1	3.1
	1995	2.3	2.0	2.0	3.8

Theoretical minimum = 1 and theoretical maximum = 4.

lipid levels, condition factors and/or fecundity in fish at some pulp mill effluent-exposed sites (Hall *et al*., 1991; Hodson *et al*., 1992; Swanson *et al*., 1994; Haley *et al*., 1995), but contrasts with the lower reproductive effort and lipid levels seen in fish near other pulp mills (McMaster *et al*., 1991; Munkittrick *et al*., 1991; Gagnon *et al*., 1995). Aquatic insects are often more abundant in pulp mill effluent plumes than they are at corresponding reference sites (Anderson, 1989; Cash *et al*., 1996; Lowell *et al*., 1996; Luoma *et al*., 1997; Thomas and Munteanu, 1997), although the phenomenon is by no means universal (Cash *et al*., 1996). Aquatic insects are more abundant downstream from the Grande Prairie pulp mill than at upstream reference sites (Fraikin *et al*., 1997) and the spatial pattern of enrichment has been interpreted as being pulp mill effluent related (Cash *et al*., 1996). The observation of increased numbers of aquatic insects downstream from pulp mills is supported by experimental evidence of increased benthic biomass in replicated artificial streams receiving pulp mill effluent (Hall *et al*., 1991; Haley *et al*., 1995). The increase in insect abundance is attributable to increases in primary productivity caused by nutrients in the effluent (Hall *et al*., 1991; Haley *et al*., 1995; Podemski and Culp, 1996). When toxic substances are absent or only present in very low concentrations in pulp mill effluent, nutrient enrichment effects may overwhelm effects of toxins on biota. While nutrient enrichment from pulp mill effluent is one possible explanation for better tree swallow reproduction and larger nestlings at downstream sites in this study, an alternative explanation is that there may exist a continuum of nutrient enrichment in these rivers from upstream to downstream (e.g. Vannote *et al*., 1980) independent of pulp mill effluent.

In conclusion, the physiological effects of pulp mill effluent that have often been seen in fish were either absent or uncertain in tree swallows at the two study areas. Possible reasons for the lack of clear effects are that (1) substances in pulp mill effluent that are responsible for such effects have low potential for food chain transfer, (2) the concentrations of toxic substances originating from the pulp mills in this study may be present at such low concentrations in the river ecosystems as to preclude toxic effects or (3) tree swallows may be relatively insensitive bioindicators for some of the effects that have often been demonstrated in fish downstream from pulp mills. However, there was a trend towards better reproductive performance and larger 16 day old nestlings at downstream compared to upstream sites, probably due to nutrient enrichment and consequent higher abundance of emerged aquatic insects. Either tree swallows are poor indicators of pulp mill effluent effects on riparian birds or such effects are unlikely to occur in riparian birds. The results should not be extended to all riparian wildlife since it has been shown that a riparian fish-eating mammal, the mink (*Mustela vison*), exhibited elevated EROD activity and impaired immune function when fed a diet of fish captured immediately below the Prince Albert pulp mill effluent (Smits *et al*., 1995, 1996).

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