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REPRODUCTIVE SUCCESS AND CONTAMINANTS IN TREE SWALLOWS (*TACHYCINETA BICOLOR*) BREEDING AT A WASTEWATER TREATMENT PLANT

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Abstract—The uptake and effects of contaminants were measured in the insectivorous tree swallow (*Tachycineta bicolor*) at a wastewater treatment site. The study examined reproductive, immunological, and growth endpoints in tree swallows exposed to chlorinated hydrocarbon contaminants and to 4-nonylphenol in wastewater lagoons at the Iona Wastewater Treatment Plant, Vancouver (BC, Canada). Clutch size was significantly lower in tree swallows breeding at Iona Island in 2000 and 2001 compared to the reference site. In 2000, fledging success was significantly lower and mean mass of nestling livers was significantly higher in the tree swallows breeding at the Iona Island Wastewater Treatment Plant. Additional factors that may influence reproductive success, such as parental provisioning and diet composition, did not differ significantly between sites. Levels of 4-nonylphenol detected in sediment and insects were elevated at the Iona Island Wastewater Treatment Plant (2000: lagoon sediment 82,000 ng/g dry wt, insects 310 ng/g wet wt; 2001: lagoon sediment 383,900 ng/g dry wt, insects 156 ng/g wet wt) compared to the reference site (2000: pond sediment 1,100 ng/g dry wt, insects not sampled; 2001: pond sediment 642 ng/g dry wt, insects 98 ng/g wet wt). These results indicate that tree swallows might be a useful indicator species for exposure to 4-nonylphenol at wastewater treatment sites; however, further work is necessary to determine the extent of uptake and effects of 4-nonylphenol in riparian insectivorous birds.

Keywords—Tree swallow Nonylphenol Wastewater

INTRODUCTION

Tree swallows (*Tachycineta bicolor*) potentially are exposed to contaminants of aquatic origin through consumption of emergent aquatic insects. For this reason, as well as their readiness to occupy nest boxes and their small foraging ranges [1,2], tree swallows commonly have been used as a bioindicator species for contaminant exposure [3,4]. This study examined reproductive, growth, and immunological endpoints in tree swallows potentially exposed to environmental pollutants due to their breeding in the vicinity of a wastewater treatment plant.

The discharge of municipal and industrial effluents provides a continuous release of persistent contaminants, such as chlorinated hydrocarbons and alkylphenol ethoxylates, into the aquatic environment [5]. Alkylphenol ethoxylates are a class of nonionic surfactants used in a variety of industrial processes and household products [6]. A breakdown product of nonylphenol ethoxylate surfactants, 4-nonylphenol (4-NP), commonly is found in wastewater effluent. Certain chemicals contained in wastewater effluent and sludge may have the ability to disrupt reproductive and developmental events associated with hormonally mediated processes [7,8]. In particular, the presence of 4-NP and its ethoxylates in wastewater effluent are of concern because some of these compounds are known estrogen mimics. The chemical 4-NP binds intracellular estrogen receptors with a potency of 10^{-5} to 10^{-3} that of 17β-estradiol [9] and can disrupt endocrine function in several species [10,11].

The chemical 4-NP previously has been detected in samples of sewage solids (2.9–16.0 μg/g dry wt) [12] from the Iona Island Wastewater Treatment Plant (IIWTP) in Vancouver (BC, Canada), the contaminated site in the present study. Those sewage solids subsequently are discharged into sewage lagoons, over which tree swallows feed during the breeding season. Insects that emerge from sludge-containing lagoons may be exposed to 4-NP, primarily during the larval stage of development through contact with contaminated sediment and pore water. It was predicted, therefore, that tree swallows breeding at IIWTP could be exposed to 4-NP, polychlorinated biphenyls, and organochlorine pesticide residues through consumption of emergent aquatic insects. Although much research on the potentially deleterious effects of 4-NP has focused on aquatic organisms, very little is known regarding the exposure and effects of 4-NP on terrestrial species, such as insectivorous birds. This study hypothesized that contaminant exposure could contribute to altered physiological functions and parental behavior in tree swallows, which in turn could affect breeding success and nestling quality.

MATERIALS AND METHODS

Study sites

From April to July 2000 and 2001, breeding populations of tree swallows were monitored at two sites in the Greater Vancouver area. The reference site selected was the Serpentine Wildlife Area ([SWA]; 49°13′N, 122°51′W), a freshwater wet-

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land managed for migrating waterfowl by Ducks Unlimited Canada (Surrey, BC). The SWA is located to the south of Surrey, a city with a population of 370,000. It is surrounded by actively farmed agricultural land with the Serpentine River forming the northern boundary. Twenty-eight nest boxes were erected approximately 1.5 m above ground on metal poles surrounding the shallow ponds in February 2000 at SWA. At IIWTP (49°13′N, 123°12′W), 40 nest boxes were located 3 to 4 m above ground on a fence surrounding the wastewater treatment plant sewage lagoons. The IIWTP, one of five wastewater treatment plants in the Greater Vancouver Regional District, provides primary treatment to wastewater from over 600,000 people in the Lower Mainland before discharging it through a 7.5-km deep-sea outfall into the Strait of Georgia (www.gvrd.bc.ca). All field procedures were carried out under permits from the Canadian Wildlife Service, the Greater Vancouver Regional District, and the British Columbia Ministry of Environment, Lands, and Parks. Laboratory work was performed under permit from the Simon Fraser University Animal Care Committee and in accordance with Canadian Council on Animal Care Guidelines.

Reproductive performance and nestling growth

Nests were monitored to determine clutch size, hatching success, and fledging success. Data on clutch size were recorded over the course of three breeding seasons (2000–2002), and hatching and fledging success were examined in 2000 and 2001. Clutch initiation date was recorded as the first day of egg laying for each nest, and hatch date was recorded as the day the majority of eggs hatched. Replacement clutches, defined as new clutches laid in boxes from which previous clutches were lost, were noted but not included in the analyses of reproductive success. Reproductive performance was defined for all nests as the clutch size (number of eggs laid), hatching success (proportion of laid eggs that hatched), fledging success (proportion of nestlings that fledged), and breeding success (proportion of eggs from which chicks fledged). At 9 and 16 d of age, the mass of all nestlings was recorded (±0.1 g) using a digital scale in 2000 and a 50-g Pesola spring scale in 2001. Age of the female parent was classified as second year or after second year, according to the criteria described by Hussell [13].

Nestling immune status and body composition

In 2000, T-cell–mediated immunity was assessed in chicks at 9 d of age, using the phytohaemagglutinin skin test [14,15] on two chicks per nest. Thickness of the right and left wing webs (patagia) of each chick was measured (±0.001 mm) with spring-loaded calipers (model 304–196, Dyer, Lancaster, PA, USA). The right wing web was injected intradermally with 30 μl of phosphate-buffered saline, and the left wing received 60 μg of phytohaemagglutinin (Sigma-Aldrich, Oakville, ON, Canada), a plant protein and T-cell mitogen, in 30-μl phosphate-buffered saline. Patagium thickness was measured a second time 24 ± 3 h later. Each measurement was made three times and the mean value was used in statistical analysis. Due to nonindependence of observations, measurements were averaged for the two chicks sampled per nest.

At 16 d of age, one randomly selected nestling per nest was sacrificed via intramuscular injection of anesthetic (30 μl each Rompun; Bayer, Toronto, ON, Canada; and Ketalean MTC Pharmaceuticals, Cambridge, ON, Canada). Immediately postdissection, the wet weight of the liver was recorded in 2000 and 2001, as well as the weight of the bursa, spleen, thymus, paired testis, and kidney in 2000.

Provisioning rates and diet composition

In 2001, the rate at which nestlings were fed was determined by half-hour observations of randomly selected nests. Because videotapes of tree swallow nest boxes show that 100% of trips made into boxes during chick-rearing involve food delivery [16], the number of visits to the nest by either parent during the half-hour observation period were recorded as feeding trips. Visiting rates were recorded when chicks were 4, 7, 9, and 12 d old. The length of all feeding visits also was recorded with a stopwatch. Weather conditions, including temperature, wind (low, moderate, high), cloud cover, and rain, were recorded on all days on which observation took place. All observations were conducted between 7:00 AM and 5:00 PM. Morning and afternoon observations were taken on alternating days at the two sites to minimize the effect of time of day on number of feeding visits.

The composition of nestling diet was analyzed in 2001 through the use of 34-gauge copper-wire ligatures placed around the throats of 8- to 10-d-old nestlings to prevent them from swallowing food delivered by adults [17]. Ligatures were applied to all nestlings in the selected nests for 30 to 50 min. During this period, the nests were observed to ensure that at least 6 to 8 feeding trips occurred. The exact number of feeding trips was recorded. Food items collected from all nestlings in one nest were combined to form a single bolus. All diet items from the boluses were identified to order except Diptera, which were classified as suborder Nematocera or other Diptera. The length of all insects was measured to the nearest 1 mm. The wet mass of all food items collected in each order or suborder also was recorded. Insects were placed in hexane-acetone–rinsed jars, frozen at −20°C, and sent for 4-NP analysis.

Nest quality

Nest quality was evaluated in 2001 by estimating nest cup volume and total nest volume. Nest cup volume and total nest volume were calculated using the following formulas, described by Lombardo [18]:

$$\text{Total nest volume} = LWD - V_c \quad (1)$$

where L = nest length, W = nest width, D = nest depth, and V_c = nest cup volume, and

$$\text{Nest cup volume} = ([4/3][\pi a^2 b]/2) \quad (2)$$

where a = cup radius and b = cup depth

All parameters of each nest were measured within 48 h of clutch initiation and then again 2 to 3 d posthatching.

Sample collection

Using an Eckman dredge at IIWTP, two sediment samples were collected in both December 1999 and July 2001 from each of three locations: An active wastewater sewage lagoon, a previously active (dredged) sewage lagoon, and a freshwater pond next to the wastewater plant. At SWA, two sediment samples were collected in both July 2000 and July 2001 from one shallow freshwater pond.

In the summer of 2000, at IIWTP, a sample of flying insects was collected from the edges of the lagoons using sweep nets, placed in hexane-rinsed jars, and stored at −20°C for 4-NP analysis. Items not likely to be consumed by tree swallows, such as flightless beetles (Coleoptera) and large moths (Lep-

idoptera), were excluded from the sample [2]. In 2001, all insects were collected by the ligature method as described above.

Livers from dissected 16-d-old nestlings were stored in hexane-rinsed glass jars at −20°C and sent for 4-NP analysis. Nestling carcasses collected in 2000 similarly were frozen and pooled by site for chlorinated hydrocarbon (polychlorinated biphenyl [PCB] and organochlorine pesticide) analysis.

Analysis of nonylphenol and its ethoxylates

In 2000, sediment, insect, and 16-d-old nestling liver samples (one pooled sample per site for each medium) were assayed for 4-NP and 4-*t*-octylphenol at the National Water Research Institute (Burlington, ON, Canada). Tissue samples were soxhlet-extracted, subjected to gel permeation chromatography to remove lipids, derivatized using acetic anhydride and pyridine, and analyzed by selected ion-monitoring gas chromatography–mass spectrometry. Sediment samples were soxhlet extracted, back extracted with aqueous potassium carbonate, derivatized with acetic anhydride, and cleaned up using silica gel column chromatography. Extracts then were analyzed using selected ion monitoring gas chromatography–mass spectrometry.

In 2001, sediment, insect, and 16-d-old nestling liver were assayed for nonylphenol and its ethoxylates at the Institute of Ocean Sciences (Sidney, BC, Canada). Homogenized samples were spiked with a suite of internal standard surrogates. A solvent mixture of acetone:CH_3CN:dichloromethane then was added to the sample. After sonication, the vial was centrifuged at 1,000 rpm for several minutes and the supernatant mixture removed. The remaining sample was extracted twice more with 2×10 ml of the solvent mixture used above. The extracts then were combined and evaporated down to virtual dryness on a hot plate. The sample extract was cleaned using solid-phase extraction columns and then loaded in the solid-phase extraction column using 6 ml of hexane. The hexane eluant was collected and saved. Sample was extracted from the solid-phase extraction column using 10 ml of dichloromethane:acetone and the cleaned extract was evaporated to almost dryness on a hot plate under a gentle stream of nitrogen. Extracts were analyzed by liquid chromatography electrospray ionization mass spectrometry.

In order to ensure comparability of results between laboratories, two sediment samples were analyzed independently for 4-NP at both the Institute of Ocean Sciences and the National Water Research Institute. Results from the interlab comparison showed very similar values. The reported concentrations of 4-NP were 2.81 and 2.46 ng/g in the first sediment sample and 0.22 and 0.21 ng/g in the second sediment sample from the Institute of Ocean Sciences and the National Water Research Institute, respectively. Standard reference material and blanks also were included in all sample analyses for quality-assurance purposes.

Analysis of chlorinated hydrocarbons

In 2000, 16-d-old tree swallow nestling carcasses (one pooled sample per site) also were analyzed for chlorinated hydrocarbons at the National Wildlife Research Center (Hull, QC, Canada). Samples were analyzed for 56 PCBs congeners and 21 organochlorine pesticides. For a list of all congeners and pesticides analyzed, see [19]. Between 1.5 and 3.0 g of homogenized nestling tissue was ground with sodium sulfate to obtain a free-flowing mixture. This mixture was poured into a 2.1-cm internal diameter chromatography column and eluted with 200 ml of dichloromethane/hexane. The extracts were reduced to less than 5 ml using a rotary evaporator, spiked with an internal standard chlorobenzene/PCB solution, and diluted to 10 ml with dichloromethane/hexane. Extracts then were cleaned up and analyzed by gas chromatography–mass spectrometry.

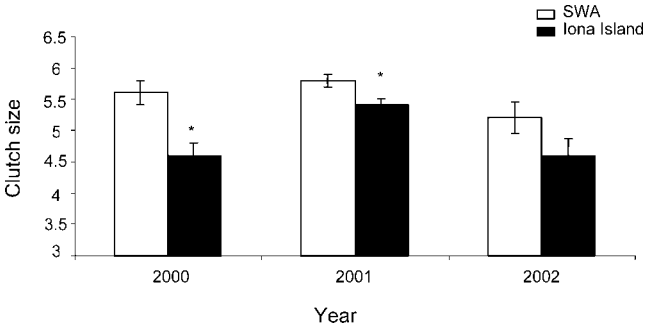

Fig. 1. Clutch sizes at Iona Island Wastewater Treatment Plant (Richmond, BC, Canada) and Serpentine Wildlife Area ([SWA]; Surrey, BC, Canada) in the years 2000, 2001, and 2002. Values are mean ± standard error (*$p < 0.05$)

Statistical analysis

All statistical analysis was performed using either SAS Version 8.0 or JMP Version 4.0 (SAS Institute, Cary, NC, USA). The level of significance for all statistical tests was set at $p < 0.05$. Data sets were tested for normal distributions using the Shapiro-Wilks test for normality. Non-normally distributed variables were transformed to common logarithms to meet normality requirements for parametric analysis. Where the condition of normality could not be met, nonparametric tests were used. Correlations of endpoints with continuous variables (date, brood size, nestling mass) and with dichotomous variables (site, sex, female age) were tested using analysis of variance or analysis of covariance, when data sets met conditions of normality, or Wilcoxon two-sample test for nonparametric analysis. Hatching, fledging, and breeding success were compared between sites using chi-square tests. Data sets for number of visits, length of visits, and nest volume were found to be normally distributed and were analyzed using the Students *t*-test. Chi-square analysis was used to determine the effects of site on nest box occupancy and differences in diet composition for the three major groups of insects consumed.

RESULTS

Reproductive performance and chick growth

Clutch initiation date did not differ significantly between sites for first broods in either year (2000; $F_{1,29} = 1.33$, $p = 0.26$, 2001; $F_{1,35} = 2.12$, $p = 0.15$). Clutch sizes were significantly smaller at IIWTP compared to SWA in the first two years (2000; $Z = 3.16$, $p < 0.002$, 2001; $Z = -1.99$, $p < 0.05$) but not in 2002 ($Z = -1.46$, $p < 0.15$; Fig. 1). Though hatching success was slightly lower at IIWTP in 2000, no significant difference was detected between sites in either year ($p > 0.05$; Table 1). Mean fledging success was significantly lower at IIWTP compared to SWA in 2000 but not in 2001 (2000; $\chi^2 = 7.49$, $p < 0.01$, 2001; $p > 0.20$), when excluding all depredated nests. Seven nests were lost to predation at IIWTP, six nests of which were depredated during the nestling stage. Because this study was interested in the effects of contaminants

Table 1. Reproductive performance in tree swallows at contaminated (Iona Island Wastewater Treatment Plant [IIWTP], Vancouver, BC, Canada) and reference (Serpentine Wildlife Area [SWA], Surrey, BC, Canada) sites during 2000 and 2001

	2000		2001	
Nest parameter	SWA	IIWTP	SWA	IIWTP
Nest box occupancy	14/28 (50.0%)	17/40 (42.5%)	25/28 (89.3%)	20/39 (51.3%)
Percentage of 2nd year females (%)	65	35	37.5	35.0
Nests abandoned	0	0	4	0
Nests depredated	1	2	0	7
Hatching success (%)[a]	92	80	90	93
Fledging success (%)[a,b]	86	68	93	91
Breeding success (%)[a,b]	80	54	84	84

[a] Mean values.
[b] Including nests from which at least one chick fledged.

on success, depredated nests were not included in the analysis. Overall breeding success was significantly lower at IIWTP in 2000 (2000; $\chi^2 = 18.86$, degrees of freedom [*df*] = 1, $p < 0.001$; Table 1). No difference was found between sites in nestling mass at 9 or 16 d in either year ($p > 0.10$ for all).

Although second-year females made up a larger proportion of breeding females at SWA compared to IIWTP in 2000, female parent age did not differ significantly between sites either year ($p > 0.06$). Female age was unrelated to clutch size, hatching success, and breeding success ($p > 0.30$ for all).

Immune status and body composition

For both absolute and relative measures ($p > 0.40$ for both), there was no difference in phytohaemagglutinin (PHA)-induced wing inflammation between sites. Neither the absolute thickness (thickness after PHA injection) nor the relative (after PHA injection–before injection) wing inflammation measure were related to brood size ($p > 0.40$ for both) or nestling mass ($p > 0.15$ for both).

Livers from 16-d-old nestlings were significantly heavier at IIWTP than at SWA ($F_{2,19} = 19.66$, $p < 0.0001$) in 2000. In 2001, livers from nestlings collected from IIWTP were again heavier; however, this difference was not significant ($F_{2,19} = 3.73$, $p = 0.07$). In bursa, spleen, thymus, paired testis, or kidney mass, there was no difference found between sites.

Provisioning rates and diet composition

The number of feeding visits did not differ significantly between sites at any time sampled during the chick-rearing period ($p > 0.21$ for all ages). Also, there was no significant difference in length of feeding visit ($p > 0.22$ for all ages).

A total of 1,175 food items from 13 boluses at SWA and 644 food items from 13 boluses were obtained from tree swallow nestlings at IIWTP. The length of the invertebrates contained in the boluses predominantly ranged from 3 to 6 mm, with the exception of the insects from the order Odonata, which had a mean length of 32 mm. Insects from the suborder Nematocera by far were the most numerous items fed to tree swallow nestlings (Fig. 2). They accounted for 60.3 and 62.3% of the total biomass at SWA and IIWTP, respectively. Terrestrial insects and other aquatic insects were much less important. Other numerically important prey included insects from the order Odonata and other Diptera. The mass of diet items belonging to Nematocera, Odonata, or other Diptera delivered to nestlings was not affected by site ($p = 0.44$). Diet items with high calcium content obtained from nestling boluses included mollusk shells, bones, and unidentifiable items. These nonliving items accounted for 4.5% of the total bolus mass at SWA and 0.2% of the food mass at IIWTP.

The biomass of food delivered per chick did not differ significantly between sites (Students *t*-test, $t = -0.672$, $df = 25$, $p = 0.51$). However, there was a slightly higher mass of food delivered per feeding trip at SWA when compared to IIWTP (Students *t*-test, $t = -2.080$, $df = 25$, $p = 0.05$).

Nest structure

Mean nest volumes were 675 ± 32 cm^3 and 292 ± 67 cm^3 at SWA and IIWTP, respectively. Nests built at SWA were significantly larger in volume than nests built at IIWTP ($t = -4.350$, $df = 34$, $p < 0.01$). Visually, nests at SWA were made of a much tighter grass weave than were those at IIWTP.

4-NP and its ethoxylates

Levels of 4-NP were highest in two sediment samples collected in 2000 and 2001 from an active evaporation pond at IIWTP (Table 2). In 2000, 4-NP was detected in a sample consisting of insects collected by sweep net at IIWTP. No insect sample was collected from SWA in 2000 for comparison. In 2001, 4-NP also was detected in insect samples collected using ligatures from both IIWTP and SWA, with higher

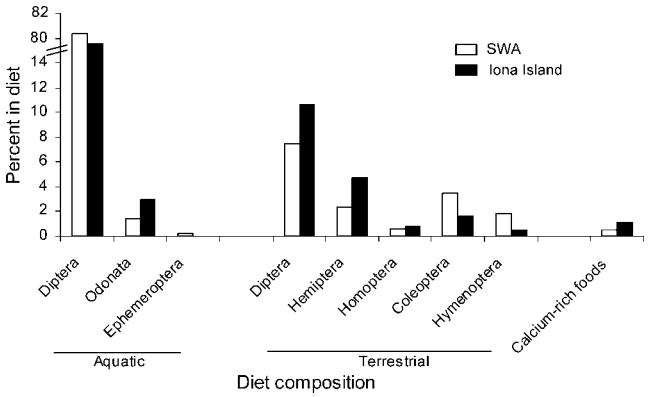

Fig. 2. Composition of food items found in boluses from tree swallow nestlings. Data are expressed as the number of food items in the order divided by the total number of food items. Samples with less than 0.1% by mass were not included in the chart. These include Neuroptera, Arachnid, aquatic Diptera (other than Nematocera), and items that could not be identified. Iona Island Wastewater Treatment Plant (Richmond, BC, Canada); Serpentine Wildlife Area ([SWA], Surrey, BC, Canada).

Table 2. Mean 4-nonylphenol (4-NP) residues and minimum detection limits (MDL) in sediment (ng/g dry wt), tree swallow nestling livers (ng/g wet wt), and insect samples (ng/g wet wt) from Iona Island Wastewater Treatment Plant ([IIWTP], Vancouver, BC, Canada) and Serpentine Wildlife Area ([SWA], Surrey, BC, Canada). ND = nondetectable; — indicates no samples collected

	2000				2001			
	SWA		IIWTP		SWA		IIWTP	
Medium	4-NP	MDL	4-NP	MDL	4-NP	MDL	4-NP	MDL
Sediment								
Active lagoon	—	—	82,000	18.8	—	—	383,900	211.2
Inactive lagoon	—	—	400	0.3	—	—	186,450	60.2
Freshwater pond	1,100	500	65	1.5	642	7.1	611	5.5
Insect	—	—	310	10.0	98	52.4	156	80.5
Liver	ND	10.0	ND	10.0	38	30.1	29.5	27.2

levels found in diet items from IIWTP (Table 2). Despite the presence of higher 4-NP in sediment and insect samples from IIWTP, 4-NP was below detectable levels in nestling livers collected in 2000. In 2001, 4-NP was detected at relatively low levels at both sites (Table 2).

PCBs and chlorinated hydrocarbons

Total PCB levels in 16-d-old tree swallow carcasses were more than an order of magnitude greater at IIWTP than at SWA (Table 3). The PCB 153 constituted 13.5% of the sample at IIWTP and 33% of the sample at SWA. Four other congeners were present at levels $\geq$ 5.0 ng/g at Iona only.

Ten of 21 chlorinated hydrocarbon compounds were detected in tissue samples from both sites. Most of the organochlorine pesticide residue was comprised of 1,1-dichloro-2, 2-bis(*p*-chlorophenyl)ethylene (IIWTP 98%, SWA 93%). The three other organochlorine pesticides detected above trace levels in tissues from at least one site were oxychlordane, *p,p′*-DDD, and heptachlor epoxide.

DISCUSSION

Reproductive performance

In general, breeding parameters for tree swallows at SWA were typical of those reported in other studies, whereas those for IIWTP were lower. For example, Robertson et al. [1] noted a mean clutch size of 5.4 eggs among 28 tree swallow populations across North America, and McCarty and Secord [20] reported a mean of 5.6 eggs for 2,958 tree swallow clutches in 15 studies.

Hatching rates did not differ between sites (>80% for both sites) and this parameter was similar to the mean hatching rate of 86.9% for tree swallows reported in a review of seven noncontaminated North American sites [1]. Mean fledging success in 2000 was significantly lower at Iona and also was lower than values reported for reference sites elsewhere. Robertson et al. [1] noted a mean fledging rate of 83% among 18 reference populations, and Bishop et al. [4] considered fledging rates above 75% to be comparable to those seen in tree swallows at noncontaminated sites.

Table 3. Polychlorinated biphenyl ([PCB]; ng/g wet wt) and organochlorine pesticide ([CHC]; ng/g wet wt) residues in pooled 16-d-old tree swallow whole bodies (minus liver and testis tissue) from the Iona Island Wastewater Treatment Plant ([IIWTP], Vancouver, BC, Canada) and Serpentine Wildlife Area ([SWA], Surrey, BC, Canada; 2000). The top five PCB congeners and organochlorine pesticides with the highest concentrations detected are listed in the table

Compound[a]	SWA	IIWTP
Sum of PCB congeners	6.0	104.0
PCB 153	2.0	14.0
PCB 138	2.0	12.0
PCB 118	1.0	9.0
PCB 101/90	Tr[b]	6.0
PCB 180	1.0	5.0
Sum of CHCs	71.0	49.0
p,p-DDE	66.0	48.0
p,p-DDD	2.0	Tr[b]

[a] DDE = 1,1-dichloro-2, 2-bis(*p*-chlorphenyl)ethylene; DDD = 1,1-dichloro-2, 2-bis(*p*-chlorphenyl)ethane.
[b] Tr = trace.

Body composition

The 16-d-old tree swallows from Iona Island had significantly heavier livers than birds at SWA, and elevated liver mass has been associated with chemical exposure in other avian species. For example, Fox [21] noted a positive relationship between liver mass in herring gulls (*Larus argentatus*) and exposure to chlorinated hydrocarbons. Mallard ducks (*Anas platyrhynchos*) fed diets containing crude oil [22] or reared on oil sands wetlands [23] experienced dose-dependent increases in liver weight. Because PCB, organochlorine pesticide, and nonylphenol levels were relatively low in nestling tissue at both sites in the present study, it is not likely that these chemicals alone reached the toxic levels reported by Talmage [6]. It is possible that the mixture of chemicals contained in the sewage lagoons could have contributed to higher liver mass in tree swallows chicks at Iona.

Provisioning rates and diet composition

Although site differences in certain reproductive parameters were consistent with higher contaminants levels at IIWTP, establishing a credible causal relationship between contaminants and adverse effects on the tree swallow population is difficult, because a number of factors besides chemical exposure are able to impact growth, reproduction, and survival.

Nestling diet composition was assessed to test the possibility that differences in reproductive performance between sites were due to differences in the types of prey consumed by swallows. Adults at both sites fed nestlings a limited number of taxa, with Nematocera being the most abundant type of prey. The diet of adult tree swallows has been reported to be similar to that of nestlings; therefore, it is likely that Nematocera also composed the majority of items consumed by breeding adults [24].

Because a previous study reported that a significant de-

crease in the number of feeding trips by parent birds was observed following organophosphorous exposure in tree swallows [16], the present study recorded the number and length of parental feeding trips at various nestling ages. No difference was found in number of feeding trips between sites. The mass of insects delivered to the nestlings also was measured to test the possibility that difference in fledging success between sites was due to different levels of food consumption. The amount of food delivered to nestlings may not be a good indicator of total food availability, because adults may adjust their delivery rate to compensate for low food abundance in response to hunger signaling from their young. Neither the mass of food delivered per chick nor the food-delivery rate differed between SWA and IIWTP during the periods sampled. It should be noted that the food-delivery rate was based on a sample size of 26 boluses and that the sampling periods represented only a small fraction of nestling life.

Nest structure

Besides the potential for direct estrogen-mimicking effects of alkylphenols in nestlings, it is possible that exposure to contaminants could affect growth and survival indirectly through alteration of parenting abilities. A previous study reported abnormal nest-building behavior in tree swallows exposed to PCBs, compounds which affect a variety of physiological endpoints, including endocrine endpoints [25]. In that study, tree swallows in contaminated areas built smaller nests of lower quality compared with those in uncontaminated areas. Nest building is an important component of parental effort in birds, and previous studies have reported that nest quality is an important determinant of reproductive success in tree swallows [18]. Both the amount and type of nest material and insulation have thermoenergetic effects [26]. The nests at SWA had a significantly larger volume than those at IIWTP. As shown in previous studies, poor nest-building skills would be consistent both with lower reproductive performance and higher contaminant exposure at Iona Island.

Exposure of tree swallows to 4-NP and other contaminants

Levels of 4-NP in sediment from a wastewater lagoon at IIWTP (means, 2000 = 82,000 ng/g; 2001 = 383,900 ng/g) were within the range of values reported for sewage sludge at wastewater treatment plants [27]. Sediment 4-NP at SWA (means, 2000 = 1,100 ng/g; 2001 = 642 ng/g) was higher than reported river sediment values [28], possibly due to run off from application of agricultural chemicals on surrounding fields or leaching directly into the ponds [29]. This is the first study to measure 4-NP in insects consumed by tree swallows, and thus the first to indicate that tree swallows could be exposed to this contaminant through their diet. In 2000, 4-NP was not detected in collected tree swallow livers and was detected in low levels in 2001, with similar levels found in birds from both sites. The lack of elevated 4-NP levels in the nestling livers from IIWTP could be due to rapid metabolism of nonylphenol or storage in other tissues [30]. It is interesting that levels of 4-NP detected in sediment samples from the reference site were elevated to the same extent as those of the freshwater pond located next to the wastewater plant. This may indicate that 4-NP contamination is distributed widely throughout the environment rather than being confined to the vicinity of point sources. Most studies on the occurrence of 4-NP have focused on monitoring in sewage treatment plant effluent and digested sludge [21].

Total PCB levels were higher in nestlings from IIWTP; however, the levels of PCBs detected in nestling samples still were much lower than those reported to cause adverse effects in birds [20,31]. No difference in hatching or fledging success was evident in tree swallows exposed to PCBs in wetlands of the Great Lakes and St. Lawrence River Basin, Canada, with maximum levels detected of 5,469 ng/g total PCBs in nestling whole bodies [4]. Impaired reproductive success, demonstrated by low egg hatchability and high rates of nest abandonment, was evident in tree swallows breeding at the highly contaminated Hudson River (NY, USA) [20]. Tree swallow nestlings that fed on insects from the Hudson River contained total PCB concentrations ranging from 3,710 to 62,200 ng/g [31].

CONCLUSION

This was the first attempt to investigate physiological and reproductive endpoints in a free-living passerine species exposed to 4-NP and other chemicals present in a wastewater treatment sewage lagoon. Lower reproductive success during the early breeding stage and higher nestling liver weight were observed in tree swallows at the wastewater treatment site. However, there was little difference in 4-NP levels in tree swallow livers between the wastewater treatment and reference sites. The PCB concentrations were an order of magnitude greater in nestling carcasses from IIWTP compared to SWA, but still less than those found in tree swallows at other sites where no adverse effects were reported. No clear association was found between nonylphenol exposure and depressed tree swallow reproduction, immunocompetence, or growth and development at the study sites. On the other hand, this study was not designed to measure subtle effects of chemicals in tree swallow adults that failed to breed or in nestlings that did not survive to 16 d. It is possible that these very individuals had the highest contaminant load.

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