

## REPRODUCTIVE SUCCESS AND CONTAMINANTS IN TREE SWALLOWS (*TACHYGINETA 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

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](http://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.





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 5 nondetectable; — indicates no samples collected

Medium	2000				2001			
	SWA		IIWTP		SWA		IIWTP	
	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 oxychlorane, *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% other In review 2088 ln[ 5510(bothgon(hyrganbhyr287(3er)s9ihyr319(2

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 <sup>a</sup>	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 <sup>b</sup>	6.0
PCB 180	1.0	5.0
Sum of CHCs	71.0	49.0
<i>p,p'</i> -DDE	66.0	48.0
<i>p,p'</i> -DDD	2.0	Tr <sup>b</sup>

<sup>a</sup> DDE 5 1,1-dichloro-2, 2-bis(*p*-chlorophenyl)ethylene; DDD 5 1,1-dichloro-2, 2-bis(*p*-chlorophenyl)ethane.

<sup>b</sup> Tr 5 trace.

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  $\pm$  82,000 ng/g; 2001  $\pm$  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  $\pm$  1,100 ng/g; 2001  $\pm$  642 ng/g) was higher than reported river sediment values [28], possibly due to runoff 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].

