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Polybrominated diphenyl ethers and multiple stressors influence the reproduction of free-ranging tree swallows (*Tachycineta bicolor*) nesting at wastewater treatment plants

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HIGHLIGHTS

- We assessed relative influences of PBDEs & multiple stressors on avian reproduction.
- Avian reproduction differed at waste water treatment plants and the reference site.
- Onset of egg laying and egg size was influenced by PBDE exposure via the outflow.
- Current PBDE exposure did not influence reproductive productivity of these birds.
- Reproductive success was influenced by lay date, ambient temperature, and predation.

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ABSTRACT

Reproductive success of birds is influenced by maternal factors, ambient temperatures, predation, food supply, and/or exposure to environmental contaminants e.g., flame retardants (FRs). Reproduction of tree swallows (*Tachycineta bicolor*) was compared among waste water treatment plants (WWTPs) and a reference reservoir in Ontario, Canada (2007–2010), to determine the importance of exposure to polybrominated diphenyl ether (PBDEs) FRs within a complex contaminant cocktail, relative to natural and biological factors known to influence avian reproduction. The birds primarily consumed insects emerging from the reference reservoir and WWTP outflows, where effluent mixed with receiving waters. FR egg concentrations were dominated by 5 PBDE congeners ($\sum_5$PBDEs): 2,2′.4,4′,5-pentabromodiphenyl ether (BDE-99), 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47), 2,2′4,4′,6-pentabromodiphenyl ether (BDE-100), 2,2′,4,4′,5,5′-hexabromodiphenyl ether (BDE-153), and 2,2′,4,4′,5,6′-hexabromodiphenyl ether (BDE-154), with much lower concentrations of decabromodiphenylether (BDE-209), hexabromocyclododecane (HBCDD), and novel FRs. Although higher than $\sum_5$PBDEs, polychlorinated biphenyls (PCBs) egg concentrations were unlikely to affect the swallows' reproduction. Clutch size and timing, fledging, breeding success, and predation, varied significantly among sites, generally being poorer at WWTP1 and better at WWTP2. The early reproductive stages were sensitive to some FRs at measured concentrations. The $\sum_5$PBDEs, maternal age, and minimum ambient temperatures predicted onset of egg laying in the most parsimonious statistical model, and there were positive relationships between egg size and HBCDD or BDE-209 concentrations. However, there were no significant correlations with any reproductive measures, individual BDE congeners or low concentrations of novel FRs, in this first such report for novel FRs and wild birds. Tree swallows are passerines, and passerines may differ from birds of prey in their reproductive sensitivity to PBDE exposure: lay date, minimum temperatures, and predation, but not PBDE exposure, predicted reproductive productivity. Overall, there was some influence of the PBDEs available in these WWTP outflows on early reproductive parameters, but not reproductive output, of these passerines.

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1. Introduction

Flame retardants (FRs) are used in a wide variety of industrial and domestic applications, and are ubiquitous in the environment. A recent estimate of the annual worldwide production of brominated FRs (BFRs) is 200,000 tons (Dominguez et al., 2011; Segev et al., 2009), with tetrabromobisphenol A (TBBPA), hexabromocyclododecane (HBCDD), and polybrominated diphenyl ethers (PBDEs), accounting for the largest production volumes of BFRs (Dominguez et al., 2011). Penta- and octa-BDEs, no longer manufactured or imported into North America, continue to enter the environment through the disposal and degradation of existing products (Ward et al., 2008). HBCDD and PBDEs are known to bioaccumulate (Luo et al., 2010); concentrations of these FRs have been measured in the tissues of free ranging birds, including mid-trophic passerines such as European starlings (*Sturnus vulgaris*) (Chen et al., 2012, 2013), and raptorial peregrine falcons (*Falco peregrinus*) at the highest trophic level (e.g., Fernie and Letcher, 2010; Guerra et al., 2011) of the Laurentian Great Lakes in North America.

Several studies involving captive birds in laboratory settings have revealed that exposure to PBDEs and HBCDD at environmentally relevant concentrations can alter the reproductive success and/or behaviors of these birds (Winter et al., 2013; Eng et al., 2012, 2013; Fernie et al., 2009, 2011; Marteinson et al., 2010; van den Steen et al., 2009a). In these studies, captive birds were housed under ideal conditions and provided food ad libitum. To date, studies specifically examining the effects of BFRs in the environment on free-ranging birds are uncommon, and have focused on birds of prey that may be more sensitive to the potential effects of chemical exposure than other birds (e.g., McKernan et al., 2009). Peregrine falcons experienced a decrease in average brood size for females with increasing concentrations of ΣPBDE in eggs (Johansson et al., 2009), suggesting that reproduction in this species may be influenced by PBDEs. Additionally, Henny et al. (2009) originally found a negative relationship between productivity and ΣPBDEs (>1000 ng/g ww) in the eggs of ospreys (*Pandion haliaetus*), but this relationship was not apparent in a subsequent study (Henny et al., 2011).

Wild birds are subject to multiple anthropogenic and natural stressors, including chemical exposure, that may influence their reproductive success. Exposure to some FRs has advanced or delayed the timing of egg laying (e.g., Eng et al., 2013; Fernie et al., 2009, 2011), and generally, birds that lay their eggs earlier in the breeding season have better reproductive success, laying more eggs and fledging more young (Dunn et al., 2011; Stutchbury and Robertson, 1988). The reproductive success of many birds, including small passerine birds, may be profoundly influenced by daily ambient temperatures (Dunn and Winkler, 1999; Hussell, 2003) and food availability (Martin, 1987; Perrins, 1991), that are closely associated with maternal body condition, clutch size, laying date, and breeding success (e.g., Goodburn, 1991; Houston et al., 1983; Pietiäinen and Kolunen, 1993). Maternal age may also influence avian reproductive success, with younger females having poorer success (Stutchbury and Robertson, 1988). Predation is another major factor that may affect the reproductive success of birds (e.g., Weidinger, 1996), particularly small passerines.

Tree swallows are small, mid-trophic, migratory passerine birds that feed primarily on aerial insects, many of which have aquatic larvae (Dunn and Hannon, 1992; Larsson, 1984; Quinney and Ankney, 1985), and feed in close proximity to their nests (≤400 m). As a result, tree swallows have been used widely as indicators of aquatic contamination (Custer, 2011). Tree swallows are known to consume insects emerging from the effluent of waste water treatment plants (WWTP) (e.g., Dods et al., 2005), a major source of flame retardants (e.g., Hale et al., 2003; Song et al., 2006). Our study objectives were to determine: 1., if there were differences in reproductive measures of birds nesting at waste water treatment plants (WWTP) compared to those at reference sites, related to WWTP effluent outflow as sources of PBDE exposure; 2., the influence of PBDE exposure on avian reproduction relative to known natural and biological factors that can alter reproduction of wild birds; 3., possible similarities and differences between passerine species, e.g., tree swallows, and birds of prey in their reproductive responses to PBDE exposure.

2. Materials and methods

2.1. Study sites

The current study was conducted with appropriate scientific and banding permits, and in accordance with the Canadian Council of Animal Care Guidelines. Breeding populations of tree swallows were monitored in southern Ontario, Canada from 2007 to 2010. The reference colony was at the Mountsberg Conservation Area (43°27′N, 80°02′W) (Fig. 1), established as a natural park in 1964, and had 49 nest boxes adjacent to a man-made reservoir. Nest boxes (n = 27) were also situated around the sewage lagoons of a WWTP in Kitchener, Ontario, Canada (WWTP1; population: 250,000), and another 71 nest boxes were located within 800 m of the effluent discharge pipe of another WWTP in Hamilton, Ontario, Canada (WWTP2; population: 500,000) (Fig. 1). All three sites were approximately 40 km apart from each other.

2.2. Reproductive, morphological, and environmental variables

We captured adult birds in their nest boxes in 2009 and 2010 only. Adult males were primarily captured during courtship and females primarily while incubating their eggs. The sex of each adult bird was based upon wing chord length, the presence of a cloacal protuberance, and the size of the brood patches (Pyle, 1997). The age of adult female birds, determined by feather coloration and wing chord length, was recorded as ‘young’ (brown plumage; in its second calendar year) or older (≥3 calendar years of age) (Pyle, 1997). The body mass of the individual bird was recorded to the nearest 0.01 g (Tanita Digital Scale Professional-Mini, Model 1479V), and the length of the tibiotarsal bone (digital Vernier calipers) and naturally-curved wing chord (metal ruler) were measured to the nearest millimeter (mm) as an indicator of structural size. In birds, condition is traditionally measured by dividing body mass by an indicator of structural size (e.g. Benson and Winker, 2005), although body mass has recently been described as a better indicator of body condition than morphometric indices (Schamber et al., 2009).

From 2007 through 2010, nest boxes used by tree swallows were monitored daily to determine clutch size (the number of eggs laid per pair), hatching success (the percentage of eggs that hatched relative to the number of eggs incubated per pair), fledging success (the percentage of hatchlings that fledged per pair), and overall breeding success (the percentage of eggs that fledged per pair). Clutch initiation date was recorded as the day the first egg was laid in the nest. One of the first three eggs in each clutch was collected, and a subset of 30 eggs from approximately 10 nests per site that successfully hatched young, were used for chemical analysis and egg measurements; these eggs were collected prior to clutch completion in order to minimize embryonic development while ensuring that nest abandonment did not occur. In 2009 and 2010, eggshell thickness (Porter and Wiemeyer, 1969) and egg volume (cm^3) (Hoyt, 1979) were determined.

Ambient temperature records were used from Environment Canada's historical weather archive (weather.gc.ca), having been recorded daily for all four years (2007–2010) from weather stations nearest to each field site (based on latitude–longitude coordinates). These weather stations were approximately 24 km from the reference site, 6 km from WWTP2, and 6 km from WWTP1. Another weather station was closer to the reference site but did not provide daily temperature records for all four years of this study.

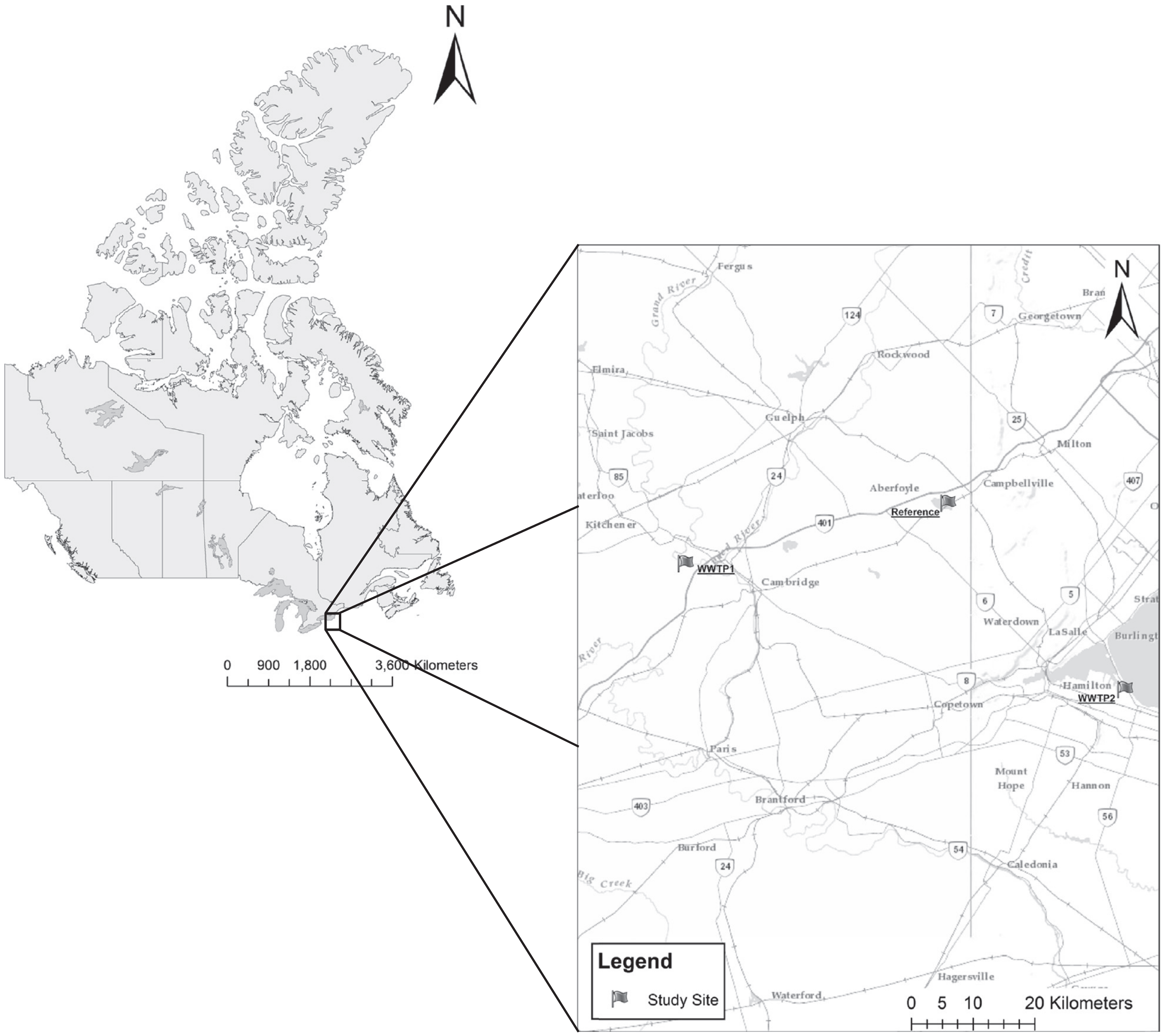

Fig. 1. Colonies of nest boxes for tree swallows were established and monitored from 2007 through 2010, at three study sites in southern Ontario, Canada: a reference reservoir, a waste water treatment plant (WWTP1) in the city of Kitchener, and WWTP2 in the city of Hamilton. Each of the sites was ~40 km distance apart.

2.3. Chemical analysis of eggs

By using one of the first 3 eggs laid in each clutch for analysis of FR, PCB and organochlorine pesticides, variations in lipid concentrations and hence contaminant concentrations were minimized since evidence suggests the occurrence of intra-clutch variation in lipids for tree swallows (Custer et al., 2010). A total of 46 PBDE congeners (BDE-1, -2, -3, -10, -15, -17, -28/PBT, -47, -49, -54, -66, -7, -71, -77, -85, -99, -100, -119, -138, -139, -140, -153, -154/BB153, -155, -170, -171/-190, -179, -180, -181, -182, -183, -188, -191, -194, -195, -196, -197, -201, -202, -203, -205, -206, -207, -208, and -209) and total HBCDD were quantified in individual eggs by the Organic Contaminants Research Laboratory (OCRL) at the National Wildlife Research Centre (NWRC), in Ottawa, Canada. Total-HBCD is the sum concentration of α-HBCDD, ß-HBCDD and γ-HBCDD, as ß- and γ-HBCDD thermally isomerize to α-HBCDD. Another 20 non-PBDE FR chemicals were quantified: 1,2,-bis-(2,4,6-tribromophenoxy)ethane (BTBPE); pentabromoethyl benzene (PBEB); 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (EHTBB); decabromodiphenyl ethane (DBDPE); 2,4,6-tribromophenyl allyl ether (TBPAE); pentabromotoluene (PBT); hexabromobenzene (HBB); pentabromobenzyl acrylate (PBBA); tetrabromo-*p*-xylene (*p*TBX); *syn*-Dechlorane Plus (*syn*-DP); *anti*-Dechlorane Plus (*anti*-DP); 1,2-dibromo-4-(1,2-dibromoethyl)-cyclohexane (α-TBECH isomer); 1,2-dibromo-4-(1,2-dibromoethyl)-cyclohexane (β-TBECH isomer); octabromo-1,3,3-trimethyl-1-phenyl indane (OBTMI); 2,2′,4,5,5′-pentabromobiphenyl (BB-101); 2,2′,4,4,5,5′-hexabromobiphenyl (BB-153); pentabromophenyl allyl ether (PBPAE); hexachlorocyclopentenyl-dibromocyclooctane (HCDBCO); 2,3-dibromopropyl tribromophenyl ether (DPTE); 2,3-dibromopropyl pentabromophenyl ether (PBP-dbpe); bis(2-ethylhexyl)-tetrabromophthalate (BEHTBP); tetrabromo-*o*-chlorotoluene (TBCT).

FR analysis methods for avian eggs have been described in detail elsewhere (Chen et al., 2012, 2013; Gauthier et al., 2009; Henny et al., 2009). Briefly, each sample was spiked with an internal standard solution involving BDE-30, BDE-156, $^{13}C_{12}$-BDE-209, $^{13}C_{10}$-*syn*-DP and $^{13}C_{10}$-*anti*-DP, with respective recoveries of 112 ± 14%, 107 ± 25%, 55 ± 12%, 93.5 ± 18% and 97 ± 21%; the volume and constituents of the solution varied among years. The sample was then subjected to accelerated solvent extraction (Dionex ASE System 200) with 50% dichloromethane–hexane. High purity nitrogen and compressed air were used to pressurize the contents of the cell. The eluant, containing the target compounds, was eluted, concentrated, and reconstituted with isooctane to a final volume of 200 μL. Lipid removal occurred by using gel permeation chromatography (GPC) on a 50 cm × 2.2 cm glass column packed with 50 g of S-X3 beads (Bio-Rad Laboratories, CA, USA). The sample was eluted with DCM/hexane (50v/50v). The first fraction (140 mL) was discarded and the second fraction (200 mL) was collected, subsequently concentrated, solvent exchanged to DCM/hexane (5v/95v) and then adjusted to a volume of approximately 0.5 mL. The FRs were determined in the isolated chemical fractions using gas chromatography-electron capture negative ionization–mass spectrometry (GC–MS(ECNI)).

For quality assurance, one method blank sample was analyzed with each batch of samples (n = 10) to check interferences and contamination. A blank subtraction was done for BDE-15, -47, -99, -100, and other FRs not discussed here. For each batch of samples, a reference material sample (double-crested cormorant, *Phalacrocorax auritus*, egg homogenate) was extracted and analyzed to ensure consistency of data acquisition. Concentrations of the FRs were recovery-corrected with their corresponding internal standard used as an internal standard method of quantification.

Concentrations of PCBs and organochlorine pesticides were analyzed by Axys Analytical Services (Sidney, British Columbia, Canada), using eggs collected in 2008 as previously described. (As will be discussed, based on the resulting concentrations in these eggs, we did not pursue such analysis with eggs from the other years of the study). Eggs were pooled, with all pools containing 3 eggs from 3 different nests per site, and then analyzed using GC–LRMS/GC–ECD. Analytical details are provided in the SI. Concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents (TEQs) were calculated by multiplying the concentration of individual PCB congeners by their respective World Health Organization (WHO) toxic equivalence factor (TEF) specific to birds (van den Berg et al., 1998). Total TEQ concentrations were calculated as the sum of TEQ for non-*ortho* and mono-*ortho* PCB congeners (CB-77, -81, -105, -114, -118, -123, -126, -156, -157, -167, -169, and -189) and reported as wet weight. Concentrations measured below the detection limit were assigned a value of half the detection limit for the purpose of calculating the TEQs. Polychlorinated-dibenzo-*p*-dioxins and polychlorinated dibenzofurans were not measured and were not included in TEQ computation.

2.4. Statistical analyses

Statistical analyses were performed using SAS 9.2 (SAS Institute, Cary, NC, USA) with first-laid clutches only (N = 388; replacement clutches were excluded). Data were tested for normality (Shapiro–Wilks test) and homogeneity of variance (F-test). Ambient temperatures were analyzed using two-factor (year, site) analysis of variances (ANOVAs). Categories for maternal age were compared among sites using Fisher's Exact test. Spearman's rank correlation analysis was used to identify possible correlations between egg FR levels and egg size or maternal physiological condition; the egg pools prevented such correlative analysis with concentrations of PCBs and organochlorine pesticides. Differences in maternal body mass and condition among sites were determined using non-parametric ANOVAs on ranked data, with Julian capture date as a covariate since physiological condition is dependent upon food abundance that varies throughout the breeding season (Quinney et al., 1986).

Data were pooled across years for reproductive endpoints since there were no differences among years (non-parametric ANOVAs: p-values ≥ 0.34). Non-parametric ANOVAs on ranked data were used to identify site differences in Julian lay date and predation (the number of eggs or chicks predated per pair). Site differences in the percentage of nests at each site that had ≥one egg and/or chick predated, were identified using Fisher's exact test. Non-parametric ANOVAs on ranked data were also used to identify significant differences among the study sites in clutch size, hatching, fledging and breeding success, with Julian lay date as a covariate. Predated nests were excluded from the analysis of hatching, fledging and breeding success. The analyses were repeated with maternal age as a factor using the sub-set of known-age birds from 2009 to 2010 (N = 143), since age can affect reproductive success in this species (Dunn et al., 2011; Stutchbury and Robertson, 1988). Factors were sequentially removed from the ANOVAs when not significant. If there were no significant interactions, the analysis was repeated using main effects only and post hoc Scheffé's tests performed to identify differences among the three sites. The level of significance for all statistical tests was $p \leq 0.05$.

2.5. Statistical modeling: Akaike's Information Criterion (AIC)

To understand the potential influences of various biological and environmental factors including PBDE exposure via WWTP outflow, on reproduction of the tree swallows, multivariate analytical techniques were used to analyze two independent datasets. Both data sets included the same dependent reproductive variables (clutch initiation date, clutch size, hatching, fledging and breeding success), and independent variables known to predict or influence those reproductive measures: the Σ_5PBDE egg concentrations which constituted ~80% of the measured in ovo FR burden (KJ Fernie and RJ Letcher, Environment Canada, unpublished data); minimum ambient temperatures in May when egg laying occurs, and in June when hatching occurs and chicks fledge from the nest; and the occurrence of predation (yes/no) of eggs or chicks. In addition, the "learning" dataset, involving 192 data points collected in 2009 and 2010, included maternal age and body mass as predictive variables. The "validating" dataset excluded the maternal variables and the data were collected for 4 years (2007–2010). To avoid pseudo-replication, data from the validating dataset were eliminated that were present in the learning dataset, allowing the models' predictive outcomes to be reliably tested. As a result, the validating dataset contained 292 unique data points.

All possible subsets of models were tested for the reproductive variables. By using the information theoretic model comparison (ITMC) technique, an Akaike's Information Criterion (AIC) value for each model was calculated from the log likelihoods obtained from fitting the linear regressions. Instead of using the standard AIC, the small-sample bias correction form (AIC_c) was used since it converges with the standard AIC value as the sample size increases (Burnham and Anderson, 2004). The formula used to calculate the AIC_c was:

$$AIC_c = -2LL + 2K + 2K\left(\frac{k+1}{n-K-1}\right) \quad (1)$$

where: LL = log likelihood, K = # of parameters and n = sample size.

Since the actual AIC_c value is less important than the change in the AIC_c value between different models, the difference (Δi) between the best model (lowest AIC_c value) and model *i* was calculated. The "best" model will have a delta AIC_c equal to 0, and represents the information lost if model *i* was used instead of the "best" model (Burnham and Anderson, 2004). Generally from a statistical perspective, if $\Delta i < 2$, the models being compared are too similar to be ranked by the AIC_c value (or Δi), and the most parsimonious model should be selected (Anderson et al., 2000). However, the model's predictive ability is a better determinant of biological outcomes, and not simply selecting the best model based on statistical criteria alone (Guthery et al., 2005). Thus, the models developed with the smaller data set were cross-validated using the larger data set, and the parsimonious models were evaluated against a stronger model that included additional variables. In our study, we investigated the top eight models ranked according to their ΔAIC_c values regardless of whether the ΔAIC_c exceeded 2.0. The best models are presented in Tables 2 and 3 for each of the 5 dependent reproductive variables of interest allowing for a comparison of the strongest and most common predictive factors influencing the reproductive measures.

3. Results

3.1. PBDE, HBCDD, PCB and pesticide exposure and site differences

At each site, the penta-BDEs had the highest in ovo concentrations and included in descending order, BDE-99, -47, -100, -153, and -154 ($\sum_5$PBDEs), with relatively lower levels of HBCDD (Table 1). There were significant site differences in the in ovo concentrations of $\sum_5$PBDEs and HBCDD (p-values ≤ 0.0411), with the in ovo concentrations of Σ_5PBDEs greater for the birds nesting at WWTP1 than at the other two sites ($p < 0.05$), and the in ovo HBCDD levels significantly greater at the two WWTP sites than the reference site (Table 1). Concentrations of novel flame retardants were also detected (PBBA, BTBPE, α- and β-TBECH, BEHTBP, TBCT, HBB, PBT, BB-101, *syn*-DP, TBPAE, PBEB, and pTBX) at 10 to 100-fold lower (mostly <3 ng/g ww) than the mean $\sum_5$PBDEs' concentrations; these results are presented and discussed in detail elsewhere (KJ Fernie and RJ Letcher, Environment Canada, unpublished data).

In the 2008 egg pools, the in ovo concentrations of lipids, *p,p′*-DDE, $\sum$chlordanes, mirex, dieldrin, and heptachlor epoxide, were similar among the study sites (p-values ≥ 0.07) (Table 2). However, there were significant differences across the three sites in the concentrations of $\sum$PCBs ($p = 0.001$) and TEQs ($p = 0.02$) (Table 2). The $\sum$PCB concentrations in egg pools from WWTP2 were significantly greater ($p \leq 0.02$) than from WWTP1 and the reference site, with similar concentrations between the latter two sites. The WWTP2 egg pools also had significantly greater calculated TEQ values than those from WWTP1 ($p = 0.03$), but they were comparable to values for the reference egg pools (Table 2).

3.2. Reproductive measures

Although adult tree swallows returned to each site within the first week of April, the start of egg laying differed among sites ($n = 388$ $F_{2,385} = 13.47$, $p < 0.0001$) and began earlier for the birds at the two WWTPs than at the reference site (post-hoc p-values ≤ 0.05) (Table 1). Clutch size overall also varied among the study sites ($n = 382$ $F_{2,381} = 7.93$, $p = 0.0004$) (raw means presented in Table 1), and was influenced by clutch initiation date ($F_{1,381} = 28.10$, $p < 0.0001$), variably so among the sites (lay date ∗ site $F_{2,381} = 7.96$, $p = 0.0004$). Egg measures were similar across the 3 sites, although there were positive associations between egg volume and increasing in ovo levels of HBCDD ($n = 54$, Spearman's rho = 0.32, $p = 0.0189$) and BDE-209 ($n = 63$, Spearman's rho = 0.25, $p = 0.0445$). There were no significant correlations of the onset of egg laying, clutch size, or egg measures, with the concentrations of individual PBDE congeners, including BDE-99, or novel flame retardants.

Excluding any predated nests, there were significant differences among the study sites for fledging success ($N = 309$ $F_{2,308} = 7.84$, $p = 0.0005$) and breeding success ($N = 314$ $F_{2,313} = 9.11$, $p < 0.0001$), but not hatching success ($N = 328$ $p = 0.11$) (Table 1). Fledging success was also significantly influenced by when egg laying began ($F_{1,308} = 4.75$, $p = 0.03$). Pairs at WWTP1 and the reference site had poorer breeding success than those at WWTP2, and those birds at WWTP1 fledged fewer chicks per pair than at WWTP2 (p-values < 0.003). There were no significant correlations between hatching, fledging, and breeding success, and the in ovo concentrations of the individual PBDE congeners, including BDE-99, and novel flame retardants.

3.3. Maternal age and physiological condition

Maternal age, but not body mass or condition, significantly differed among the three sites (Fisher's exact test; $p = 0.0004$), with the reference site having a smaller proportion of older females (67%) than at WWTP1 (100%) and WWTP2 (94%) (Table 1). While maternal age and the condition index were weakly and positively correlated ($n = 191$, Spearman's rho = 0.17, p-value = 0.02), there was no significant

Table 1
Reproductive measures of tree swallows exposed to multiple anthropogenic and natural stressors including brominated flame retardants in wastewater treatment plant outflow, southern Ontario, Canada (2007–2010).[a] Concentrations of flame retardants are measured as ng/g ww.

	Reference			WWTP1			WWTP2		
	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM
Lipids (%)	27	7.0	0.4	38	6.1	0.2	68	6.3	0.2
Σ_5PBDEs[b,c]	25	83.6^A	15.7	37	590.1^C	81.2	65	205.5^B	15.4
ΣHBCDD[d]	16	0.6^B	0.1	25	2.2^A	0.7	46	2.2^A	0.8
Clutch initiation date	71	139^A	1	69	136^B	1	248	133^B	0
Clutch size[e]	68	4.79	0.16	69	4.57	0.15	245	4.87	0.06
Predated eggs[f]	71	0.70^A	0.20	67	0.84^A	0.19	248	0.27^B	0.06
Predated chicks[f]	67	0.40^A	0.16	67	0.57^B	0.16	244	0.13^A	0.04
Hatching success (%)[g]	53	81.7	3.9	44	79.1	4.8	217	87.8	1.6
Fledging success (%)[h]	53	84.9^A	5.0	44	75.9^B	6.4	217	93.8^A	1.6
Breeding success (%)[i]	53	75.1^A	4.9	44	68.1^A	6.1	217	85.9^B	1.7
Females ≥ 2 yr (%)	33	67^A		29	100^B		98	94^B	
Female body weight (g)	33	22.3	0.3	29	22.0	0.3	98	22.0	0.2
Female condition[j]	33	0.199	0.003	29	0.196	0.003	98	0.193	0.002
Minimum ambient temperatures:									
April	67	0.8^A	0.03	69	2.3^B	0.03	245	4.1^C	0.02
May	67	5.2^A	0.08	69	7.1^B	0.07	245	8.9^C	0.4
June	67	11.1^A	0.04	69	13.1^B	0.04	245	15.5^C	0.02

[a] Raw means and standard errors about the means (SEMs) are presented for initial nests only, and further exclude predated nests for hatching success, fledging success and breeding success; post-hoc statistical means and SEMs are presented for ambient temperatures. Means with different capitalized letters are significantly different at $p < 0.05$.
[b] Sum of the 5 PBDE congeners with the highest concentrations (BDE-47, BDE-99, BDE-100, BDE-153, and BDE-154).
[c] Chemical concentrations are measured in the whole egg homogenate of individual eggs (ng/g ww).
[d] Total of all HBCDD isomers.
[e] Total number of eggs laid per pair excluding the egg collected for analysis. The significant interaction term precludes determination of significant differences in site-specific post-hoc comparisons.
[f] The number of eggs or chicks per pair that were predated.
[g] The proportion of eggs that hatched per pair excluding nests with predated eggs.
[h] The proportion of nestlings that fledged per pair excluding nests that had chicks predated.
[i] The proportion of eggs that developed into young that fledged per pair excluding all predated nests.
[j] Female (maternal) condition index was calculated as body mass divided by wing chord length (the latter data are not presented).

Table 2
Concentrations (ng/g ww) of organochlorine pesticides and polychlorinated biphenyls (PCBs) measured in tree swallow egg pools collected in 2008 from two waste water treatment plants (WWTPs) and a reference reservoir in southern Ontario. All of the egg pools consisted of 3 eggs, one egg collected from each of 3 nests per site in 2008 only; the number of egg pools per site reflects the number of nests at that site from which eggs were available. TEQs were calculated according to WHO (van den Berg et al., 1998) using non-detection values set to half of the detection limit.

	Reference (N = 2)				WWTP1 (N = 3)				WWTP2 (N = 10; di-CBs N = 9)			
	Mean	SEM	Max	Min	Mean	SEM	Max	Min	Mean	SEM	Max	Min
Lipid (%)	8.1	0.3	8.5	7.8	8.0	0.2	8.4	7.9	7.8	0.4	8.9	4.9
$\sum$PCBs	411.5	189.5	601.0	222.0	286.7	81.3	435.0	155.0	2541.0	558.0	6730.0	1210.0
$\sum$hexa-CBs	160.4	72.7	233.0	87.7	104.6	27.8	155.0	58.9	960.5	210.2	2560.0	464.0
$\sum$mono-CBs	.	.	.	.	.	.	.	.	.	.	.	.
$\sum$di-CBs	.	.	.	.	.	.	.	.	0.9	0.1	1.5	0.4
$\sum$tri-CBs	3.3	0.8	4.0	2.5	3.3	0.6	4.2	2.2	29.7	3.3	53.4	17.3
$\sum$tetra-CBs	13.8	3.2	16.9	10.6	16.7	3.1	21.3	10.8	103.0	14.2	202.0	52.3
$\sum$penta-CBs	43.6	4.6	48.2	39.0	56.0	10.1	66.9	35.9	330.1	52.5	717.0	152.0
$\sum$hexa-CBs	160.4	72.7	233.0	87.7	104.6	27.8	155.0	58.9	960.5	210.2	2560.0	464.0
$\sum$deca-CBs	2.1	1.2	3.3	0.9	0.6	0.0	0.7	0.6	1.6	0.2	2.4	1.0
$\sum$hepta-CBs	142.1	82.0	224.0	60.1	77.8	31.8	140.0	34.8	873.2	220.1	2500.0	322.0
$\sum$octa-CBs	40.7	22.6	63.2	18.1	24.4	11.0	46.1	9.9	229.3	58.2	659.0	84.3
$\sum$nona-CBs	5.8	2.3	8.1	3.4	2.8	0.7	4.2	1.8	14.4	2.7	35.1	7.1
TEQ-WHO	0.0056	0.0036	0.0091	0.0020	0.0045	0.0004	0.0052	0.0039	0.0146	0.0026	0.0306	0.0071
p,p′-DDE	387.0	54.0	441.0	333.0	311.7	146.8	604.0	141.0	628.5	62.6	1060.0	402.0
Mirex	29.5	4.1	33.5	25.4	12.9	4.8	22.5	7.4	30.2	5.6	57.3	10.4
$\sum$Chlordanes	11.3	0.7	12.1	10.6	15.9	4.3	22.5	8.0	15.1	1.4	22.1	9.9
HE	2.0	0.2	2.2	1.7	4.3	2.2	8.8	1.6	2.6	0.3	4.5	1.6
Dieldrin	1.0	0.0	1.0	1.0	1.7	0.5	2.1	0.7	3.2	1.4	15.7	0.7

effect of maternal age on clutch size, predation, hatching, fledging or breeding success ($p > 0.05$). There were also no associations between maternal condition, clutch size, and in ovo $\sum_5$PBDE concentrations, or with maternal variables and the date of their capture ($p > 0.09$).

3.4. Predation

Predation rates of nests significantly differed among the three study sites (Fisher's exact test; $p = 0.002$), with more nests predated at WWTP1 (19% of nests) and the reference site (17%) than at WWTP2 (7%). Predation of eggs ($n = 386$ $F_{2,385} = 10.26$, $p < 0.0001$) and chicks ($n = 378$ $F_{2,377} = 7.53$, $p = 0.0006$) per pair showed a similar pattern of significant site differences, with more eggs predated at WWTP1 and the reference site than WWTP2, and more chicks predated at WWTP1 than WWTP2 (p-values ≤ 0.05) (Table 1).

3.5. Ambient temperature

The minimum temperatures from April to July differed among sites ($F_{2,1980} = 99.72$; $p < 0.0001$) and years ($F_{1,1980} = 4.18$; $p = 0.0409$), being colder at the reference site than the other two sites, and colder at WWTP1 than WWTP2 (all p-values < 0.0001) (Table 1). Reproductive success weakly improved with warmer temperatures ($n = 354$–370, Spearman's rho-values: 0.11–0.17, p-values ≤ 0.04).

3.6. PBDE exposure, environmental and biological factors, and reproduction

AIC models were used to identify the variable influences of the birds' exposure to PBDEs and natural factors measured in this study, on key reproductive parameters. In the smaller 'learning' data set of 2009 and 2010, maternal age, minimum ambient temperatures in May, and the birds' exposure to $\sum_5$PBDEs (in ovo concentrations) predicted the initiation date of egg laying, and these factors accounted for 23% of the variability in lay date ($R^2 = 0.23$) (Table 3). The date of clutch initiation weakly predicted clutch size in both data sets ($R^2 = 0.10$) (Tables 3, 4), and the timing of egg laying in combination with minimum ambient temperatures and the occurrence of predation, strongly predicted hatching, fledging and breeding success in 2009 and 2010 ($R^2 > 0.71$) (Table 3). This pattern was consistent with the results involving the larger 'validation' data set from 2007 to 2010: the timing of egg laying, predation, and to a lesser extent ambient temperatures, but not in ovo $\sum_5$PBDE concentrations, were the most important predictors of all 3 measures of reproductive success ($R^2 > 0.68$) (Table 4).

Several results were statistical artifacts. In 2009–2010, minimum ambient temperatures in June supposedly predicted egg laying in May, and predation of chicks in June predicted the number of eggs laid by each female in May (Table 3). Similarly, in 2007–2010, predation of chicks in June predicted clutch size in May (Table 4). These results are implausible, biologically meaningless, and should be disregarded.

4. Discussion

In this study, there were significant differences in reproductive measures among tree swallows at waste water treatment plants and the reference site. The exposure of the swallows to $\sum_5$PBDE concentrations and other environmental contaminants occurred through their feeding on aquatic-emerging insects from the WWTP outflow and reference reservoir. The start of egg laying by the birds was predicted by their exposure to $\sum_5$PBDEs in conjunction with maternal age and minimum ambient temperatures, and this was evident in the most parsimonious model only. In turn, the timing of egg laying, minimum ambient temperatures and predation, but not the $\sum_5$PBDE concentrations, predicted the reproductive output of the tree swallows. There were no correlations with the reproductive measures of the tree swallows and their exposure to individual PBDE congeners or novel FRs at the concentrations measured in this study. While the birds had greater $\sum$PCB concentrations and PCB-TEQ values at WWTP2 than the other two sites, their reproductive success at this site was generally similar or better than that of the other birds. Moreover, their PCB concentrations and TEQ values are below those previously reported for tree swallows in which these measures had little if any effect on their reproduction (Neigh et al., 2006a, 2006b; Jayaraman et al., 2009).

The $\sum_5$PBDE and HBCDD concentrations in the eggs of the current tree swallows significantly varied among the sites, with the eggs from WWTP1 more highly contaminated with $\sum_5$PBDE concentrations than at WWTP2 or the reference site. However, it is unlikely that these $\sum_5$PBDE site differences are related to differences in food availability among the sites, since the $\sum_5$PBDE egg burdens were not associated with maternal body weight or condition, a reflection of maternal exposure in passerines (Dauwe et al., 2006). In the current study, the in ovo $\sum_5$PBDE concentrations, in combination with maternal age and ambient temperatures, predicted the start of egg laying by the tree

Table 3
Summary AIC_c statistics for top candidate regression models from all possible combinations of 8 factors (7 for the Julian egg laying date model) using the smaller 'learning' data set of 187 tree swallows monitored in 2009 and 2010. Models predict egg laying date, clutch size, hatching success, fledging success and breeding success of TRES at 187 monitored nest boxes in 2009 and 2010. Ranked model represents the best AIC model ranked according to the value only, but selected as advised by Guthery et al., 2005.

Model	Date egg laying began	Maternal age	Maternal body weight	Min May Temp	Min June Temp	Predation of eggs	Predation of chicks	$\sum_5$PBDE egg levels	AIC_c	ΔAIC_c	RSS	R^2	Ranked Model
Date egg laying begins	X	✓		✓	✓			✓	769.82	0	11,835.02	0.23	1
Clutch size	✓						✓		14.40	0	190.6	0.1	1
Hatching success	✓			✓	✓	✓			−699.83	1.39	3.68	0.85	7
Fledging success	✓			✓	✓	✓	✓		−528.15	1.15	9.36	0.71	3
Breeding success	✓			✓	✓	✓	✓		−686.52	0	3.92	0.86	1

swallows in 2009 and 2010 only; the initiation date of egg laying, in conjunction with other natural factors but not the egg $\sum_5$PBDE concentrations, predicted the reproductive success of these birds.

Site differences in food quality and availability may be reflected by maternal condition. In tree swallows, the condition of the birds depends on food abundance that varies throughout the breeding season (Quinney et al., 1986). Hence, maternal condition in the current study may serve as an indicator of differences in food availability among the study sites. However, maternal condition, assessed mainly during the incubation period, was similar among the sites, and there was no association between maternal condition or body weight and when the females were captured. These findings suggest that food availability was similar among the three study sites. Yet, physiological condition does not preclude differences in the timing of food availability, and food may have become abundant earlier for birds nesting at WWTP2, a site with warmer ambient temperatures early in the breeding season.

The reproductive success of many birds is profoundly influenced by the onset of laying their clutch of eggs. Generally, birds that lay their eggs earlier in the breeding season have better reproductive success, laying more eggs and fledging more chicks (Dunn et al., 2011; Stutchbury and Robertson, 1988). Ecological and environmental factors can influence the timing of egg laying. The onset of egg laying has been altered by exposure to various flame retardants (e.g., Fernie et al., 2009, 2011; Marteinson et al., 2012; Winter et al., 2013), and in tree swallows, by temperature (Dunn and Winkler, 1999; Hussell, 2003; Nooker et al., 2005). Egg laying was delayed when raptorial American kestrels (*Falco sparverius*) were exposed by diet to the flame retardant mixture DE-71 (Fernie et al., 2009) and advanced with their dietary exposure to HBCDD (Fernie et al., 2011). In passerines, the timing of egg laying was delayed in captive zebra finches (*Taeniopygia guttata*) exposed to BDE-99 as nestlings only (Eng et al., 2013), and a similar trend was seen in zebra finches exposed embryonically to BDE-99 by egg injection (Winter et al., 2013). Yet there were no changes in the timing of egg laying when captive European starlings were exposed to PBDEs (van den Steen et al., 2009a). In contrast, in the current study, egg laying began significantly earlier at the two WWTPs, where the birds experienced greater exposure to $\sum_5$PBDEs and warmer temperatures, and in 2009 and 2010, was predicted by ambient temperatures, maternal age, and in ovo PBDE concentrations in the one model.

Clutch size in birds may also be influenced by chemical exposure, ambient temperatures, food availability, maternal age and maternal condition. Clutch sizes were smaller when captive American kestrels were exposed to β-TBECH (Marteinson et al., 2012) or as embryos to DE-71 (Marteinson et al., 2010), yet kestrels laid more eggs when exposed to HBCDD (Fernie et al., 2011). While clutches were smaller when zebra finches were exposed to BDE-99 as embryos via egg injection (Winter et al., 2013), clutch size was unaffected in zebra finches exposed to BDE-99 as nestlings (Eng et al., 2013), and in captive European starlings (van den Steen et al., 2009a) and American kestrels (Fernie et al., 2009) exposed to PBDEs. Similarly, despite the variation in clutch size of the tree swallows in the current study, there was no (direct) influence of PBDEs on clutch size, or correlative evidence with their exposure to BDE-99 or novel FRs. For small passerine birds like tree swallows and possibly starlings, daily temperatures and food supply influence clutch size, with correlative evidence of cooler air temperatures, lower insect (food) abundance, poorer maternal condition, and the laying of smaller clutches (Dunn and Winkler, 1999; Hussell, 2003; Perrins, 1991). The clutch sizes of the current tree swallows varied among the study sites and were primarily predicted by when they began to lay their eggs—being advanced at the two WWTP sites where birds experienced greater exposure to not only PBDEs, but also warmer temperatures than at the reference site.

Egg size in birds has been sensitive to chemical exposure, including BFRs (e.g., Fernie et al., 2009, 2011; Marteinson et al., 2010). For European starlings exposed to PBDEs, egg size increased compared to control birds (van den Steen et al., 2009a). For blue tits, van den Steen et al. (2009b) found a positive correlation between egg size and in ovo concentrations of $\sum$PBDEs and other measured organochlorines, with $\sum$PBDE levels in the blue tits (27 ± 5 ng/g lw to 49 ± 5 ng/g lw) being somewhat higher than in the tree swallows of the current study (means: 5.8 to 36 ng/g lw). The egg size of the tree swallows in the present study was not associated with the in ovo concentrations of $\sum$PBDEs, individual BDE congeners, or novel FRs, but was positively correlated with in ovo concentrations of BDE-209 and HBCDD. While concentrations of BDE-209 occur in measurable quantities in the eggs of free-ranging birds (e.g., Johansson et al., 2011; Muñoz-Arnanz et al., 2011; Guerra et al., 2012), no studies to date have reported any association between reproductive parameters and BDE-209. The correlative increase in egg size of the tree swallows with HBCDD concentrations, contrasts with the smaller eggs laid by captive American kestrels exposed to environmentally relevant levels of HBCDD Technical Mixture (Fernie et al., 2011) or to DE-71 (Fernie et al., 2009; Marteinson et al., 2010).

Table 4
Summary AIC_c statistics for top candidate regression models from all possible combinations of 6 factors (5 for the model regarding the initiation date of egg laying). Models predict the initiation date of egg laying, clutch size, hatching success, fledging success and breeding success of tree swallows at 240 nest boxes monitored from 2007 to 2010 ('validation' data set), excluding those nest boxes reported in Table 2. Ranked model represents the best AIC model ranked according to the value only, but selected as advised by Guthery et al., 2005.

Model	Date egg laying began	Min May Temp	Min June Temp	Predation of eggs	Predation of chicks	$\sum_5$PBDE egg levels	AIC_c	ΔAIC_c	RSS	R^2	Ranked Model
Date egg laying begins	X		✓				1232.76	0.95	21,001.3	0.07	2
Clutch size	✓						39.18	0.92	323.43	0.1	2
Hatching success	✓		✓	✓			−1128.1	0.47	5.39	0.87	3
Fledging success			✓	✓	✓		−868.1	0	13.37	0.68	1
Breeding success	✓			✓	✓		−1108.5	0.74	5.77	0.85	4

Exposure to PBDEs has been associated with reduced brood size in wild peregrines (mean Σ_6PBDE: 3,800 ng/g lw) (Johansson et al., 2009) and reduced productivity in wild ospreys (>1000 ng/g ww) in some studies (Henny et al., 2009 but see Henny et al., 2011). In captive studies with American kestrels, another raptor species, pipping success was reduced following in ovo injections with PBDEs (McKernan et al., 2009), as were hatching and reproductive success when adult kestrels were exposed by diet (Fernie et al., 2009) but not in ovo (Marteinson et al., 2010) to DE-71, or by diet to HBCDD (Fernie et al., 2011) or β-TBECH (Marteinson et al., 2012). In the Fernie et al. (2009) study, the reduced reproductive success of the kestrels was also related to their delayed egg laying, and the kestrels laid eggs with mean PBDE concentrations (3 ± 0.5 controls; 289 ± 33 ng/g ww and 1131 ± 95 ng/g ww for kestrels exposed to DE-71) that bracketed those found in the tree swallow eggs in this study (maximal mean: 590 ± 81 ng/g ww). In the current study, there was no evidence that exposure to PBDEs, HBCDD, or the novel FRs, at the concentrations measured in the eggs of the tree swallows, were correlated with their hatching, fledging or overall breeding success, despite differences in these parameters among the study sites. Similarly, following in ovo injections or nestling exposure, there was no evidence of changes in the hatching or fledging success of captive zebra finches that were exposed to BDE-99 (Eng et al., 2013; Winter et al., 2013) or TBECH (Currier et al., 2013). These findings suggest that passerines and raptors may differ in their reproductive responsiveness to PBDEs, and this bears further study.

Reproductive success of wild birds is also influenced by predation. Tree swallows are small birds that are subject to predation by reptilian, mammalian and avian species (Finch, 1990), including house sparrows (*Passer domesticus*). Nest predation (nests having ≥1 egg and/or chick predated) varied among the current study sites, with nearly one in five nests predated at WWTP1 (19%) and the reference site (17%). More specifically, there was greater predation of eggs at these same two sites, and of chicks at WWTP1 where birds had the highest in ovo PBDE concentrations. However predation, not exposure to PBDEs, significantly predicted reproductive success of the swallows. The WWTP1 tree swallows experienced longer foraging trips and associated absences from their nest boxes (KJ Fernie, Environment Canada, pers. observations), and this site was immediately adjacent to a suburban neighborhood supporting a large population of house sparrows.

Ambient temperatures, food availability, and maternal factors are other multiple stressors that can influence reproduction in wild birds, and are often interrelated. The reproduction of wild birds has been reduced in association with cooler temperatures that reduce food availability, and in turn, influence female condition (Hipfner et al., 1999; Pietiäinen and Kolunen, 1993; Weidinger, 1996). However, in the current study, female condition and body mass were similar among the sites, and did not influence reproductive parameters of the swallows. Warmer temperatures occurred at the two WWTP sites, especially WWTP2 closest to Lake Ontario. These warmer temperatures significantly influenced reproduction of the swallows. Maternal age predicted when egg laying began in the most parsimonious statistical model, and this likely reflects the larger proportion of young female swallows at the reference site where egg laying began later than at the two WWTP sites. However, maternal age did not influence hatching, fledging or breeding success of the swallows, contrasting with another study in which younger females had poorer reproductive success (Stutchbury and Robertson, 1988).

5. Conclusions

Multiple factors from anthropogenic and natural sources contributed to changes in the reproductive success of the wild passerine birds in the current study. The exposure of the free-ranging tree swallows to flame retardants, particularly PBDEs, available in the effluent outflow of urban municipal WWTPs from which they fed, was greater at these sites than the reference reservoir. The tree swallows were also exposed to novel FRs, PCBs and various organophosphate pesticides at the WWTPs and reference reservoir; none of the swallows' reproductive measures were correlated with the individual PBDE congeners or novel FRs, and at the measured concentrations, the PCBs and pesticides were unlikely to have altered their reproduction. However, at the in ovo $\sum_5$PBDE concentrations measured in the tree swallows in the current study, the $\sum_5$PBDEs appeared to have only influenced the initiation date of egg laying in conjunction with maternal age and ambient temperatures at the time and only in the most parsimonious statistical model. Thereafter, hatching, fledging and breeding success may have varied among the study sites and were predicted by when egg laying began, ambient temperatures and predation, but not maternal factors or PBDE in ovo concentrations, despite significant differences in these factors among the study sites. The results of the current study suggest that the exposure of wild passerines to PBDEs in sewage effluent outflow, at levels measured in this study, in conjunction with other biological and environmental factors, is sufficient to influence when egg laying begins, but not their reproductive success. Moreover, the timing of egg laying and/or egg size, but not reproductive success, of wild passerine species appear to be sensitive to PBDE exposure at concentrations measured here, similar to other passerines (Eng et al., 2013; van den Steen et al., 2009a, 2009b; Winter et al., 2013) and suggesting possible differences in reproductive responsiveness compared to birds of prey (Fernie et al., 2009; Henny et al., 2009; Johansson et al., 2009). Overall there was some influence of the PBDEs available in the WWTP effluent outflows on early reproductive parameters of these small passerine birds, but not on their reproductive output at levels measured in the current study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2013.10.090.

References

Anderson DR, Burnham KP, Thompson WL. Null hypothesis testing: problems, prevalence and an alternative. J Wildl Manage 2000;64:912–23.
Benson A-M, Winker K. Fat-deposition strategies among high-latitude passerine migrants. Auk 2005;122:544–57.
Burnham KP, Anderson DR. Multimodel inference: understanding AIC and BIC in model selection. Sociol Methods Res 2004;33:261–304.
Chen D, Letcher RJ, Martin PA. Flame retardants in eggs of American kestrels and European starlings from southern Lake Ontario region (North America). J Environ Monit 2012;14:2870–6.
Chen D, Martin P, Burgess NM, Champoux L, Elliott JE, Forsyth DJ, et al. European starlings (Sturnus vulgaris) suggest that landfills are an important source of bioaccumulative flame retardants to Canadian terrestrial ecosystems. Environ Sci Technol 2013;47:12238–47.
Currier HA, Letcher RJ, Williams TD, Elliott JE. An assessment of in ovo toxicity of the flame retardant 1,2-dibromo-4-(1,2-dibromoethyl) cyclohexane (TBECH) in the zebra finch. Bull Environ Contam Toxicol 2013;91:455–9.

Custer CM. Swallows as a sentinel species for contaminant exposure and effect studies. Wildlife ecotoxicology: forensic approaches. New York: Springer; 2011. p. 45–91.
Custer CM, Gray BR, Custer TM. Effects of egg order on organic and inorganic element concentrations and egg characteristics in tree swallows, *Tachycineta bicolor*. Environ Toxicol Chem 2010;29:909–21.
Dauwe T, Jaspers VLB, Covaci A, Eens M. Accumulation of organochlorines and brominated flame retardants in the eggs and nestlings of great tits, *Parus major*. Environ Sci Technol 2006;40:5297–303.
Dods PL, Birmingham EM, Williams TD, Ikonomou MG, Bennie DT, Elliott JE. Reproductive success and contaminants in tree swallow (*Tachycineta bicolor*) breeding a wastewater treatment plant. Environ Sci Technol 2005;24:3106–12.
Dominguez AA, Law RJ, Herzke D, de Boer J. Bioaccumulation of brominated flame retardants. Handb Environ Chem 2011;16:141–85.
Dunn PO, Hannon SJ. Effects of food abundance and male parental care on reproductive success and monogamy in tree swallows. Auk 1992;109:488–99.
Dunn PO, Winkler DW. Climate change has affected the breeding date of tree swallows throughout North America. Proc R Soc B Biol Sci 1999;266:2487–90.
Dunn PO, Winkler DW, Whittingham LA, Hannon SJ, Robertson RJ. A test of the mismatch hypothesis: how is timing of reproduction related to food abundance in an aerial insectivore? Ecology 2011;92:450–61.
Eng ML, Elliott JE, MacDougall-Shackleton SA, Letcher RJ, Williams TD. Early exposure to 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99) affects mating behavior of zebra finches. Toxicol Sci 2012;127:269–76.
Eng ML, Williams TD, Elliott JE. Developmental exposure to a brominated flame retardant: an assessment of effects on physiology, growth and reproduction in a songbird, the zebra finch. Environ Pollut 2013;178:343–9.
Fernie KJ, Letcher RJ. Historical contaminants, flame retardants and halogenated phenolic compounds in peregrine falcon (*Falco peregrinus*) nestlings in the Canadian Great Lakes Basin. Environ Sci Technol 2010;44:3520–6.
Fernie KJ, Shutt LJ, Letcher RJ, Ritchie IJ, Sullivan K, Bird DM. Environmentally relevant concentrations of DE-71 and HBCD alter eggshell thickness and reproductive success of American kestrels. Environ Sci Technol 2009;43:2124–30.
Fernie KJ, Marteinson SC, Bird DM, Ritchie IJ, Letcher RJ. Reproductive changes in American kestrels (*Falco sparverius*) in relation to exposure to technical hexabromocyclododecane flame retardant. Environ Toxicol 2011;30:2570–5.
Finch DM. Effects of predation and competitor interference on nesting success of house wrens and tree swallows. Condor 1990;92:674–87.
Gauthier LT, Potter D, Hebert CE, Letcher RJ. Temporal trends and spatial distribution of non-polybrominated diphenyl ether flame retardants in the eggs of colonial populations of Great Lakes herring gulls. Environ Sci Technol 2009;43:312–7.
Goodburn SF. Territory quality or bird quality? Factors determining breeding success in the magpie *Pica pica*. Ibis 1991;133:85–90.
Guerra P, Fernie K, Jiménez B, Pacepavicius G, Shen L, Reiner E, et al. Dechlorane Plus and related compounds in peregrine falcon (*Falco peregrinus*) eggs from Canada and Spain. Environ Sci Technol 2011;45:1284–90.
Guerra P, Alaee M, Jiménez B, Pacepavicius G, Marvin C, MacInnis G, et al. Emerging and historical brominated flame retardants in Peregrine Falcon (*Falco peregrinus*) eggs from Canada and Spain. Environ Int 2012;40:179–86.
Guthery FS, Brennan LA, Peterson MJ, Lusk JJ. Information theory in wildlife science: critique and viewpoint. J Wildl Manage 2005;69:457–65.
Hale RC, Alaee M, Manchester-Neesvig JB, Stapleton HM, Ikonomou MG. Polybrominated diphenyl ether flame retardants in the North American environment. Environ Int 2003;29:771–9.
Henny CJ, Kaiser JL, Grove RA, Johnson BL, Letcher RJ. Polybrominated diphenyl ether flame retardants in eggs may reduce reproductive success of ospreys in Oregon and Washington, USA. Ecotoxicology 2009;18:802–13.
Henny CJ, Grove RA, Kaiser JL, Johnson BL, Furl CV, Letcher RJ. Wastewater dilution index partially explains observed polybrominated diphenyl ether flame retardant concentrations in osprey eggs from Columbia River Basin, 2008–2009. Ecotoxicology 2011;20:682–97.
Hipfner JM, Gaston AJ, Martin DL, Jones IL. Seasonal declines in replacement egg-laying in a long-lived, Arctic seabird: costs of late breeding or variation in female quality? J Anim Ecol 1999;68:988–98.
Houston DC, Jones PJ, Sibley RM. The effect of female body condition on egg laying in lesser black-backed gulls *Larus fuscus*. J Zool 1983;200:509–20.
Hoyt DF. Practical methods of estimating volume and fresh weight of bird eggs. Auk 1979;96:73–7.
Hussell DJT. Climate change, spring temperatures, and timing of breeding of tree swallows (*Tachycineta bicolor*) in Southern Ontario. Auk 2003;120:607–18.
Jayaraman S, Nacci DE, Champlin DM, Pruell RJ, Rocha KJ, Custer CM, et al. PCBs and DDE in tree swallow (*Tachycineta bicolor*) eggs and nestlings from an estuarine PCB superfund site, New Bedford Harbor, MA, U.S.A. Environ Sci Technol 2009;43:8387–92.
Johansson A, Sellström U, Lindberg P, Bignert A, de Wit CA. Polybrominated diphenyl ether congener patterns, hexabromocyclododecane, and brominated biphenyl 153 in eggs of peregrine falcons (*Falco peregrinus*) breeding in Sweden. Environ Toxicol Chem 2009;28:9–17.
Johansson AK, Sellström U, Lindberg P, Bignert A, de Wit CA. Temporal trends of polybrominated diphenyl ethers and hexabromocyclododecane in Swedish peregrine falcon. Environ Int 2011;37:678–86.
Larsson P. Transport of PCBs from aquatic to terrestrial environments by emerging chironomids. Environ Pollut 1984;34:283–9.
Luo X, Chen S, Mai B, Fu J. Advances in the study of current-use non-PBDE brominated flame retardants and Dechlorane Plus in the environment and humans. Sci China Chem 2010;53:961–73.
Marteinson SC, Bird DM, Shutt JL, Letcher RJ, Ritchie IJ, Fernie KJ. Multi-generational effects of polybrominated diphenylethers exposure: embryonic exposure of male American kestrels (*Falco sparverius*) to DE-71 alters reproductive success and behaviors. Environ Toxicol Sci 2010;29:1740–7.
Marteinson SC, Letcher RJ, Graham L, Kimmins S, Tomy G, Palace VP, et al. The flame retardant β-1,2-dibromo-4-(1,2-dibromoethyl)cyclohexane (β-TBECH): fate, fertility and reproductive success in American kestrels (*Falco sparverius*). Environ Sci Technol 2012;46:8440–7.
Martin TE. Food as a limit on breeding birds: a life-history perspective. Annu Rev Ecol Syst 1987;18:453–87.
McKernan MA, Rattner BA, Hale RC, Ottinger MA. Toxicity of polybrominated diphenyl ethers (DE-71) in chicken (*Gallus gallus*), mallard (*Anas platyrhynchos*), and American kestrel (*Falco sparverius*) embryos and hatchlings. Environ Toxicol Chem 2009;28:1007–17.
Muñoz-Arnanz J, Sáez M, Aguirre JI, Hiraldo F, Baos R, Pacepavicius G, et al. Predominance of BDE-209 and other higher brominated diphenyl ethers in eggs of white stork (*Ciconia ciconia*) colonies from Spain. Environ Int 2011;37:572–6.
Neigh AM, Zwiernik MJ, MacCarroll MA, Newsted JL, Blankenship AL, Jones PD, et al. Productivity of tree swallows (*Tachycineta bicolor*) exposed to PCBs at the Kalamazoo River Superfund site. J Toxicol Environ Health A 2006a;69:395–415.
Neigh AM, Zweirnik MJ, Bradley PW, Kay DP, Park DS, Jones PD, et al. Tree swallow (*Tachycineta bicolor*) exposure to polychlorinated biphenyls at the Kalamazoo River superfund site, Michigan, USA. Environ Toxicol Chem 2006b;25:428–37.
Nooker JK, Dunn PO, Whittingham LA. Effects of food abundance, weather, and female condition on reproduction in tree swallows (*Tachycineta bicolor*). Auk 2005;122:1225–38.
Perrins CM. Tits and their caterpillar food supply. Ibis 1991;133:49–54.
Pietiäinen H, Kolunen H. Female body condition and breeding of the diurnal owl *Strix uralensis*. Funct Ecol 1993;7:726–35.
Porter RD, Wiemeyer SN. Dieldrin and DDT: effects on sparrowhawk eggshells and reproduction. Science 1969;165:199–200.
Pyle P. Identification guide to north American birds, part 1. Bolinas, California: Slate Creek Press; 1997.
Quinney TE, Ankney CD. Prey size selection by tree swallows. Auk 1985;102:245–50.
Quinney TE, Hussell DJT, Ankney CD. Sources of variation in growth of tree swallows. Auk 1986;103:389–400.
Schamber JL, Esler D, Flint PL. Evaluating the validity of using unverified indices of body condition. J Avian Biol 2009;40:49–56.
Segev O, Kushmaro A, Brenner A. Environmental impact of flame retardants (persistence and biodegradability). Int J Environ Res Public Health 2009;6:478–91.
Song M, Chu SG, Letcher RJ, Seth R. Fate, partitioning and mass loading of polybrominated diphenyl ethers (PBDEs) during the municipal treatment processing of wastewater. Environ Sci Technol 2006;40:6241–6.
Stutchbury BJ, Robertson RJ. Within-season and age-related patterns of reproductive performance in female tree swallows. Can J Zool 1988;66:827–34.
van den Berg M, Birnbaum L, Bosveld ATC, Brunstrom B, Cook P, Feeley M, et al. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ Health Perspect 1998;106:775–92.
van den Steen E, Eens M, Covaci A, Dirtu AC, Jaspers VLB, Neels H, et al. An exposure study with polybrominated diphenyl ethers (PBDEs) in female European starlings (*Sturnus vulgaris*): toxicokinetics and reproductive effects. Environ Pollut 2009a;157:430–6.
van den Steen E, Jaspers VLB, Covaci A, Dauwe T, Pinxten R, Neels H, et al. Maternal transfer of organochlorines and brominated flame retardants in blue tits (*Cyanistes caeruleus*). Environ Int 2009b;35:69–75.
Ward J, Mohapatra SP, Mitchell A. An overview of policies for managing polybrominated diphenyl ethers (PBDEs) in the Great Lakes basin. Environ Int 2008;34:1148–56.
Weidinger K. Egg variability and hatching success in the Cape Petrel *Daption capense* at Nelson Island, South Shetland Islands, Antarctica. J Zool 1996;239:755–68.
Winter V, Williams TD, Elliott JE. A three-generational study of in ovo exposure to PBDE-99 in the zebra finch. Environ Toxicol Chem 2013;2013:562–8.