

PRODUCTIVITY OF TREE SWALLOWS (*TACHYGINETA BICOLOR*) EXPOSED TO PCBs AT THE KALAMAZOO RIVER SUPERFUND SITE

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*A 123-km stretch of the Kalamazoo River in Michigan, was designated a Superfund site in 1990 due to historical releases of effluent containing polychlorinated biphenyl (PCB)-contaminated paper waste. Risk to bird species in the river ecosystem was evaluated using the tree swallow (*Tachycineta bicolor*) as a monitor for possible effects due to PCB exposure at two nesting locations, one in the Superfund site and one in an upstream reference location that is less contaminated with PCBs. In 2 of the 3 years of the study, clutch size at the contaminated location was 3.7 ± 1.4 and 4.8 ± 0.73 eggs per nest (mean \pm SD), which was significantly less than the clutch size at the reference location (5.0 ± 1.1 and 5.3 ± 1.1 eggs per nest). However, there were no statistically significant differences in fledging success, predicted brood size, predicted number of fledglings, or growth of nestlings between the Kalamazoo River Superfund site and an upstream reference location with lesser concentrations of PCBs in the sediments and riparian soils. Productivity and hatching success comparisons between these same sites were also not significantly different; however, the power of these conclusions was less ($p < .10$). The reduction in clutch size at the co-contaminated location could not be attributed to PCBs due to a number of confounding factors, including Co-cocontaminants, habitat structure, and food availability. Other reproductive parameters were not significantly impaired, and the size of the newly established colony at the Kalamazoo River Superfund site continued to grow over the period of the study. These site-specific observations, combined with multiple lines of evidence approach that considered results reported for the effects of both total PCBs and 2,3,7,8 tetrachlorodibenzo-p-dioxin equivalents (TEQ) on tree swallows at other locations, suggest that there were no significant population-level effects of PCBs on tree swallows at the Kalamazoo River Superfund site.*

In 1990, 123 km of the Kalamazoo River in southwest Michigan was designated a Superfund site based on the presence of polychlorinated biphenyls (PCBs) in fish, sediments, and floodplain soils. PCBs have been linked to

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adverse effects in numerous avian species (Giesy et al., 1994). These effects include reproductive dysfunction (Dahlgren et al., 1972; Britton & Huston, 1973; Lillie et al., 1974; Ludwig et al., 1993; Halbrog et al., 1998; Grasman & Whitacre, 2001; Fairbrother et al., 2004), embryonic deformities (Ludwig et al., 1993; Summer et al., 1996), and growth impairment (Lillie et al., 1974; Ludwig et al., 1993). The tree swallow (*Tachycineta bicolor*) has been frequently used as a monitor of contaminants in North American riverine and lacustrine systems, particularly to assess exposure to PCBs and their potential bioavailability, dietary, and tissue concentrations, and subsequent effects on reproduction and behavior (Ankley et al., 1993; Jones et al., 1993; Bishop et al., 1998a, 1998b, 1999; Froese et al., 1998; McCarty & Secord, 1999; Harris & Elliott, 2000; Custer et al., 2003; Martinovic et al., 2003a, 2003b). In the study on which we report here, the tree swallow was selected as a representative for passerine species exposed to sediment-derived PCBs through the aquatic food chain. The tree swallow also has a feeding range of approximately 0.1 km (McCarty & Winkler, 1999) to 5 km (Robertson et al., 1992) during the nesting period, which assures that pairs nesting near the river will feed on prey in direct contact with PCB-contaminated sediment. Tree swallows are insectivorous, typically feeding on emergent aquatic insects just above the water surface (Quinney & Ankney, 1985; Cohen & Dymerski, 1986; McCarty, 1997). Tree swallows have been used for studies, such as the one on which we report here, because they nest readily in boxes and exhibit no observer-induced abandonment (Rendell & Robertson, 1990).

Concentrations of PCBs were significantly greater in tree swallow eggs and nestlings within the Kalamazoo River Superfund site compared to concentrations in birds from an upstream reference location (Neigh et al., 2006a). The risk of PCB-induced effects was determined by comparing site-specific concentrations of PCBs in bird tissues to toxicity reference values (TRVs) determined in controlled laboratory studies with surrogate species (Neigh et al., 2006a). However, extrapolating from laboratory experiments to tissue concentrations observed in the field has inherent uncertainties (U.S. EPA, 1998). Thus, in conjunction with the tissue-based assessment of risk, this study monitored tree swallow reproductive and developmental health on the Kalamazoo River directly by measuring population-level responses, including fecundity, fertility, and weight gain, of nestling tree swallows exposed to PCBs in their diet.

MATERIALS AND METHODS

Site Details

Two sites within the Kalamazoo River floodplain were selected to study nesting tree swallows. The former Trowbridge Impoundment (TB) (Figure 1) was selected as the worst-case scenario for exposure to wildlife within the

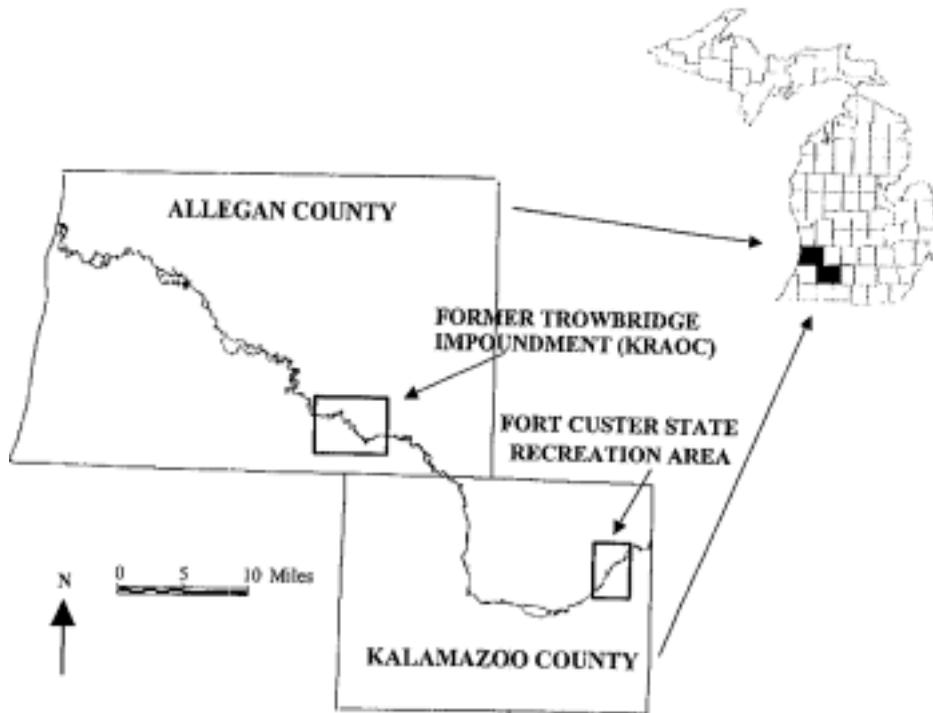


FIGURE 1. Map of the Kalamazoo River Area of Concern (KRAOC) and reference site. The boxes show the boundaries of the upstream reference location (Fort Custer State Recreation Area) and the Trowbridge Impoundment, located within the KRAOC (Neigh et al 2006a). Copyright © 2006 Society of Environmental Toxicology and Chemistry From Environmental Toxicology and Chemistry, by Neigh AM, et al. Reprinted by permission of Alliance Communications Group, a division of Allon Press, Inc.

Kalamazoo River Superfund site because it contained the greatest concentrations of PCBs and was the largest of the impoundments. The TB area is located upstream of the former Trowbridge Dam Impoundment near Allegan, MI. The study area was wholly within the Kalamazoo River Area of Concern (KRAOC), which is a U.S. Environmental Protection Agency-designated Superfund site. The TB dam was removed to its sill in 1986, which resulted in the exposure of a large and contiguous landmass of former lake bottom, on which nest boxes were established. The former Trowbridge Impoundment is located approximately 67 km downstream of a reference area and includes approximately 132 ha of former sediments, currently exposed, flanking 7 km (70 ha) of remaining impounded water. The former sediments have minimal residential or agricultural development, so the extensive floodplain remains under natural vegetation of lowland forest, wet grassland, lowland shrub, and emergent marsh.

A reference site in the Fort Custer State Recreation Area (FC) was selected because it is situated 48 km upstream of known sources of PCBs and is characterized

by lesser concentrations of PCBs. The FC site was the most similar to the TB impoundment in habitat and proximity to the river's course. Michigan State University and the Kalamazoo Nature Center have conducted nest box studies on the site for the last 30 yr, and the tree swallow population has demonstrated normal levels of reproductive success (R. Adams, personal communication).

Productivity and Attentiveness Observations

In April 2000, nest boxes were placed at both the TB and FC areas. At the FC site, nest boxes ($n = 38$) had been previously established during long-term studies, but an additional 26 boxes were added for a total of 64 nest boxes. The 2000 field season was the first year boxes were available for nesting throughout the TB area ($n = 68$), so occupancy rates at this site were expected to be less than those at the previously established FC site. For this reason, 2000 was designated a baseline year to establish nesting populations in the newly placed boxes at both FC and TB. The establishment and weathering of boxes in 2000 was intended to improve the correlation of data gathered from individuals nesting in the newly erected boxes and individuals utilizing the previously established nest boxes at the FC site.

Nest boxes were selectively placed in open/prairie grassland habitats to increase occupancy rates by secondary-cavity nesting passerine species. All boxes were placed within the floodplain, not more than 200 m from the river. Wire-mesh predator guards surrounded the entrance hole. Beginning in early April, boxes were checked every 3 d for the initiation of nest building. Once nest construction was complete, the nest was checked daily to determine date of clutch initiation, which was reported as Julian days. Within 24 h of when eggs were laid, the mass and length of eggs were recorded, and each egg was given a unique identification code with a permanent marker. Egg width was not recorded for eggs due to the difficulty of measuring this endpoint in the field and the potential for an increased rate of observer breakage. Clutch size was determined as the total number of eggs laid by a single female. Incubation was determined by touch. Warm eggs indicated incubation, whereas nests with no defending adults and cold eggs for 7 consecutive days were considered abandoned. Upon completion of the clutch, the nest was checked every few days until the end of the 14-d incubation period, at which time the nest was again examined daily to determine day of hatch. Day of hatch was also confirmed by assessing physical development of the nestling. Each nestling was examined for gross morphological abnormalities. With hatch day designated as d 0, weight of each nestling was recorded on d 3, 9, and 12 as an approximation of growth. Nestling body measurements were also used to approximate growth. On nestling d 12, the length of the entire body (tip of beak to longest rectrix), tarsus (tibio-tarsal joint to hind toe base), and unflattened wing chord were recorded. The gender of individual nestling tree swallows could not be determined because plumage coloration was similar between sexes, but when possible,

age of adult females was determined according to plumage characteristics described by Hussell (1983).

This study was conducted in three parts that included tissue concentrations (Neigh et al., 2006a), dietary exposure (Neigh et al., 2005b), and reproductive health (presented here) to determine whether tree swallows are likely to exhibit effects attributed to exposure through the food chain from the presence of PCBs in floodplain soils and in-stream sediment. The use of this site for measurement of these three lines of evidence prevented the authors from applying traditional approaches for assessing reproductive success because nests used for reproductive measurements were also used to determine tissue and dietary exposure. In this study we chose to examine nest specific measurements of exposure and reproduction to address variability between nests in a population and to report exposure based on the upper 95% confidence limit of concentrations present at the site. This type of approach involves removing eggs or individuals from all nests and may compromise some endpoints of the study, specifically brood size, number of fledglings, hatching success, and overall productivity, since the ultimate fate of an egg or nestling could not be definitively determined at the time of sampling. The study design presented here may limit the applicability of other studies to this one, but there is little influence on the study's main goal of determining whether reproductive success was different between exposed and unexposed populations at the Kalamazoo River.

The study's sampling design, in which eggs were removed after clutch completion for residue analyses, prevented the brood size (number of nestlings at hatch) and the number of fledglings from being determined directly, so a predicted brood size and number of fledglings was calculated based on reproductive success measurements. One fresh egg or one live nestling was sampled from each nest. In total, 5, 17, and 19 eggs were removed for chemical analysis from nests on the Kalamazoo River during 2000, 2001, and 2002, respectively. Predicted brood size was calculated as the product of the clutch size and the hatching success of the eggs remaining in the nest after an egg was removed. The number of fledglings was calculated as the clutch size multiplied by productivity, which was the product of hatching and fledging success. Hatching success was determined as the number of nestlings at the time of hatching per egg remaining in the nest after removal of an egg. Sampled eggs were excluded from the analyses because the viability of the eggs was unknown at sampling. Fledging success was determined as the number of nestlings at the last day of weighing divided by the number of nestlings at the time of hatch. It was assumed that all nestlings taken live would have fledged and that all nestlings at the latest nest check had fledged, unless their carcasses were found or the nest was empty before the normal fledging age of 19–21 d (Robertson et al., 1992).

Parental attentiveness of tree swallow adults was assessed during 30-min observation sessions of the nest box. The number of visits by the adults is correlated with the number of feedings (McCarty, 2002). On d 1 through 17 of

the nestling period, each box was observed for a minimum of 3 sessions to determine the approximate number of times the adults fed the nestlings. Observations took place during the most active feeding periods between 08:00 and 12:00 and between 18:00 and 21:00. In order to minimize the effect of nestling age and to avoid pseudo-replication, the average number of visits per nestling over all observations was calculated for each box. Average nestling age at both locations during nest observations were 9, 8, and 10 d for 2000, 2001, and 2002, respectively.

Statistical Analyses

Meristic data were examined by a combination of parametric and non-parametric statistical procedures, depending on the structure of the data. The normality of each sample set was assessed with the Kolmogorov–Smirnov one-sample test with Lilliefors's transformation. Data sets that were determined to have normally distributed values were compared by Student's *t*-tests, or, in the case of multiple comparisons, by one-factor analysis of variance (ANOVA). Data with nonnormal distributions and/or unequal variances were analyzed by Mann–Whitney *U*-tests or Kruskal–Wallis nonparametric tests for two or multiple samples. All data, regardless of normality and homogeneity of variance, were evaluated for interaction with cofactors based on analysis of covariance (ANCOVA). Although the assumption of normality was violated in some instances, we do not believe that ANOVA based on the ranks would have been a more reliable estimate of interaction because of the loss of discriminatory ability inherent in the ranking process (J. Newsted, personal communication). The criterion for significance used in all tests was $p < 0.05$.

In order to isolate reproductive effects induced by exposure to PCBs, measurements from some nests were excluded from analysis after a disturbance, but measurements taken before the disturbance were included. Disturbances included predation, human activities, loss of the nest box, or habitat alteration near the nest box. Several nests were excluded after predation, which was determined by the presence of house wrens near the nest box or when the nest material was disturbed. Abandoned nests were included in the calculation of hatching success, fledging success, and productivity until the point when they were abandoned.

In order to compare growth rates of nestlings over the nestling period at each location, logistic growth curves were fitted to the average nestling mass in each box across all nests. Curves based on a logistic model have been shown to be the most appropriate for describing nestling growth in tree swallows (Zach & Mayoh, 1982). Growth curves between sites were also statistically compared based on average nestling mass gain from d 3 to d 10 over the nestling period. Nestling masses were log-transformed, and the mass gain, calculated as the mass gained per day of life, was compared between sites.

RESULTS

Reproductive Success

The mean number of clutches initiated during the study was greater at the Fort Custer State Recreation Area than at the Trowbridge Impoundment. At TB there were 9, 12, and 13 clutches initiated during 2000, 2001, and 2002, respectively, and 20, 24, and 27 clutches initiated during the same 3-year period at FC. Female age was evaluated at each nest during 2001 and 2002 to address the influence of adult age on reproductive parameters such as clutch size and date of clutch initiation. There was a difference between years in the proportion of second year (SY) to after second year (ASY) females during 2001 (Mann–Whitney *U*) but not during 2002. SY females were in their first breeding season and ASY females were in at least their second breeding season. TB had a greater number of SY birds during 2001 compared to 2002 and compared to FC during both years (Figure 2). Female age was not a statistically significant cofactor for any reproductive parameters (ANCOVA).

Clutch size differed significantly between years at each site, so years were analyzed separately. Mean clutch size at FC was significantly greater than at TB during 2000 and 2002 (Mann–Whitney *U*) (Table 1). The date of clutch initiation was a significant covariate with clutch size in 2000 and 2001, but not 2002. Even when date of initiation was included as a cofactor, clutch size was still not significantly different between sites in 2001. No measures of productivity, besides clutch size, were significantly different between sites in any year. The predicted number of nestlings and predicted number of fledglings also were not significantly different between sites in any year (Mann–Whitney *U*).

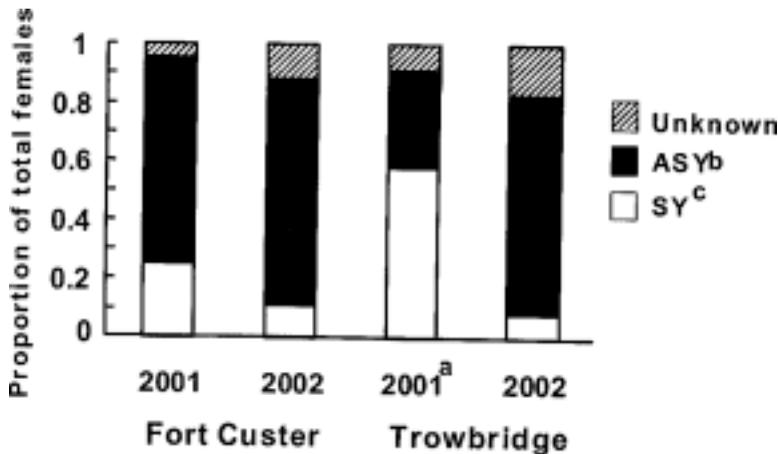


FIGURE 2. Percentage of the total population in each year represented by each female age class at the Fort Custer (FC) reference location and the Trowbridge (TB) contaminated location. a, Mean female age was significantly different from the Fort Custer reference population (Mann–Whitney *U*, $p = 0.038$). b, After second year (ASY) birds were in at least their second breeding season. c, Second year (SY) birds or subadults are in their first breeding season.

TABLE 1. Reproductive Measurements (Mean (SD)) for Tree Swallows at the Reference Site (FC) and a PCB-Contaminated Site (TB) the Kalamazoo River Area of Concern

	2000			2001			2002			All years combined						
	n	FC	TB	n	FC	TB	n	FC	TB	n	FC	TB				
Clutch size	20	5.0 (1.1)	9	3.7 (1.4) ^d	24	5.3 (0.91)	11	5.3 (0.79)	27	5.3 (1.1)	13	4.8 (0.73) ^e	71	5.2 (1.0)	33	4.6 (1.1) ^f
Hatching success ^a	20	0.77 (0.26)	9	0.68 (0.41)	23	0.77 (0.26)	10	0.83 (0.19)	17	0.71 (0.26)	13	0.64 (0.36)	60	0.75 (0.25)	32	0.71 (0.33)
Fledging success ^b	19	1.00 (0.00)	7	1.00 (0.00)	22	0.96 (0.21)	9	1.00 (0.00)	17	0.94 (0.13)	11	0.98 (0.08)	58	0.97 (0.15)	27	0.99 (0.05)
Productivity ^c	20	0.77 (0.26)	9	0.68 (0.41)	23	0.74 (0.30)	9	0.81 (0.20)	17	0.66 (0.26)	13	0.62 (0.35)	60	0.72 (0.27)	31	0.69 (0.33)
Predicted brood size	19	3.9 (1.1)	7	3.5 (1.4)	22	4.3 (1.3)	10	4.3 (1.2)	17	3.9 (1.4)	11	3.8 (1.6)	58	4.2 (1.3)	28	3.9 (1.4)
Predicted fledglings per nest	19	3.9 (1.1)	7	3.5 (1.4)	21	4.2 (1.3)	9	4.3 (1.2)	17	3.6 (1.4)	11	3.7 (1.6)	57	4.0 (1.3)	27	3.8 (1.4)

^aPercentage of eggs hatched in each nest.

^bPercentage fledged per nestling hatched.

^cPercentage fledged per egg laid.

^dSignificantly different from the FC reference population (Mann-Whitney U, $p < 0.05$).

^eSignificantly different from the FC reference population (Mann-Whitney U, $p < 0.05$).

^fSignificantly different from the FC reference population (Mann-Whitney U, $p < 0.05$).

When nests in which an egg was sampled were removed from the analyses, no results changed. Although nonnormal distribution prevented parametric tests, female age and date of clutch initiation were evaluated for covariation with reproductive parameters. Female age and date of clutch initiation described some of the variation in the data sets, but no cofactor interacted with reproductive parameters to such a degree that the significance of the test statistic changed. The majority of nest failures at FC during 2002, and over the course of the study, were due to competition by house wrens. At TB, about 57% of the nest failures were due to abandonment and 14% to predation (Table 2).

Mean egg lengths and weights are presented for each location (Table 3). Mean egg length and weight exhibited a statistically significant interaction (ANCOVA), but the interaction with clutch size was not significant. There were no significant differences between years at either site, so measurements were combined for multiple years. Average egg weight was significantly greater at TB than FC (Mann–Whitney *U*), but the average length of the eggs was not significantly different between locations.

Date of clutch initiation was significantly different among years at both sites (Kruskal–Wallis) and was significantly different between sites during 2001 and 2002 (Mann–Whitney *U*). Median clutch initiation dates were generally earlier at TB than at FC, but the range of dates over which nests were initiated was later at FC. For all years, 7 clutches at TB and 13 clutches at FC were more than ± 1 SD from the mean date of clutch initiation at a site and were designated as early or late nesters. Upon recalculation of reproductive success to exclude outlying early and late nesters, only the significance of clutch size between sites changed in 2002. Thus, differences in clutch initiation dates did not significantly affect the conclusions about other parameters.

TABLE 2. Nest Fate and Percent of Total Nests Initiated at the Fort Custer (FC) Reference Site and the Trowbridge (TB) Target Site for Nests in Which Eggs Were Laid

	2000		2001		2002	
	FC	TB	FC	TB	FC	TB
Number of successful nests ^a	19 (95%)	7 (78%)	21 (88%)	9 (75%)	17 (63%)	11 (85%)
Number abandoned ^b	1 (5%)	2 (22%)	1 (4%)	0 (0%)	0 (0%)	2 (15%)
Number predated ^c	0 (0%)	0 (0%)	1 (4%)	1 (8%)	9 (33%)	0 (0%)
Number of others ^d	0 (0%)	0 (0%)	1 (4%)	2 (17%)	1 (4%)	0 (0%)
Total number initiated	20 (100%)	9(100%)	24 (100%)	12 (100%)	27 (100%)	13 (100%)
Total number of boxes available	64	68	64	68	64	68

^aNests in which at least one nestling fledged.

^bNests containing eggs and/or nestlings but without an adult present for at least 7 d.

^cNests that had signs of disturbed nest material or had evidence of tossed or damaged eggs attributable to house wren competition.

^dNests with an unknown fate or failed due to disease.

TABLE 3. Mean (SD)^a Reproductive Endpoints for Tree Swallow Eggs and Nestlings (12-d-old) Reared at the Fort Custer State Recreation Area and at the Trowbridge Impoundment Along the Kalamazoo River Area of Concern

Parameter	Fort Custer	Trowbridge
Eggs		
<i>n</i>	71	34
Egg weight (g)	1.80 (0.02)	1.86 (0.15) ^b
Egg length (mm)	19.0 (0.8)	19.0 (0.7)
Nestlings		
<i>n</i>	13	8
Body weight (g)	22.12 (1.47)	22.37 (1.87)
Body length (mm)	93.8 (8.6)	90.8 (6.0)
Tarsal length (mm)	13.3 (1.9)	13.7 (2.7)
Wing chord (mm)	49.9 (11.0)	50.9 (5.7)

^aMean values were calculated based on the mean measurements per nest for all nests with eggs or nestlings present during the entire study period from 2000 to 2002.

^bMean was significantly different from the Fort Custer reference population (Mann-Whitney *U*, $p < .05$).

Growth and Growth Curves

Growth parameters of mean weight, tarsal length, wing chord length, and body length for nestlings sampled on d 12 were not significantly different between the 2 sites when all sample years were combined (Table 3). When growth parameters were evaluated for covariation, body length and wing chord variances were significantly associated, but this did not change the significance of mean growth parameters between sites. When nestlings were weighed repeatedly, there were few differences in average mass of nestlings between locations, except on d 13 when TB nestlings were significantly heavier than FC nestlings (Student's *t*-test) (Table 4).

Growth curves were virtually identical between sites and among years (Figure 3). Logistic growth curves yielded R^2 values of between 0.90 and 0.93. There were no statistically significant differences in mass gain between sites in any year (Student's *t*-test) or when all years were combined (Mann-Whitney *U*).

Parental Attentiveness

At the Kalamazoo River, 127 h of feeding observations were made during a 3-yr period. In order to minimize the effect of brood size on parental attentiveness, the number of visits per 30-min period was normalized to the number of nestlings in each nest. Years were analyzed separately because the mean number of visits per nestling was significantly different between years at TB (ANOVA). When the covariates time of observation, nestling age, and date of observation were factored into the model to compare among years within

TABLE 4. Tree Swallow Nestling Weight (g) Expressed as the Mean Nestling Weight in Each Nest at the Upstream Fort Custer Reference Area and the Former Trowbridge Impoundment From 2000 to 2002

Nestling age (d)	Fort Custer		Trowbridge	
	<i>n</i>	Mean (SD)	<i>n</i>	Mean (SD)
2	4	4.71 (0.46)	2	4.11 (0.14)
3	34	5.81 (1.60)	17	6.26 (1.55)
4	7	8.45 (2.18)	4	8.63 (2.78)
5	4	8.52 (1.70)	3	10.97 (2.50)
6	3	14.28 (0.92)	1	13.72 (NA) ^b
7	4	17.80 (3.96)	3	16.99 (3.12)
8	2	18.75 (1.84)	4	19.59 (2.63)
9	40	20.31 (1.97)	17	19.55 (1.77)
10	7	21.95 (2.64)	4	22.27 (1.69)
11	7	22.97 (0.94)	6	22.96 (0.98)
12	43	22.15 (2.34)	12	22.01 (1.06)
13	8	22.80 (1.16)	5	24.08 (0.60) ^c

^aSignificantly different from reference location (Student's *t*-test, *p* < 0.05).

^bNA, not available.

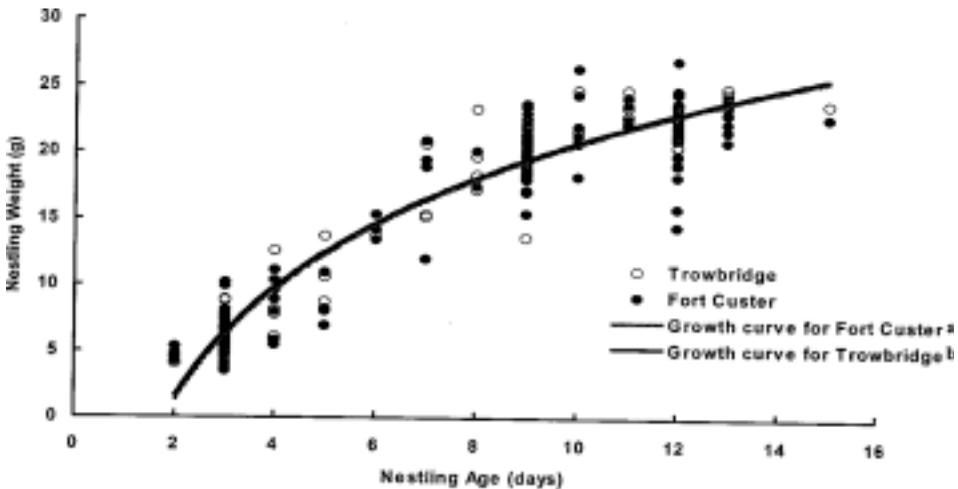


FIGURE 3. Tree swallow (TRSW) nestling growth curves for Fort Custer (FC) reference site and Trowbridge (TB) target site based on mean nestling mass for each nest box. a, Logistic growth curve defined by the line of best fit at Fort Custer, $y = 11.962 \ln(x) - 6.9272$, $R^2 = 0.93$. b, Logistic growth curve defined by the line of best fit at Trowbridge, $y = 11.898 \ln(x) - 6.6376$, $R^2 = 0.90$.

each site, 2002 remained significantly different from other years at TB (ANCOVA). The mean number of visits per nestling was not significantly different between sites for either 2000 or 2001. However, the number of visits per nestling was significantly different in 2002 (Student's *t*-test). TB had a mean

number of visits per nestling of 0.49 ± 0.37 (± 1 SD), whereas the mean number of visits for FC was 1.00 ± 0.44 (± 1 SD) in 2002. Weather does not appear to have been a factor explaining this difference, because the times and dates of observation were randomly dispersed between sites. No interactions with time, nestling age, or date of observation were significant in any analysis between sites.

Gross Morphology and Abnormalities

Upon examination of the external morphology of nestlings and contents of eggs, no external developmental abnormalities were observed at any site during any sampling year.

DISCUSSION

For select parameters, comparative examinations of receptor responses were evaluated in proximal populations of exposed versus unexposed cohorts. For this study, every effort was made to minimize confounding factors that may interact with PCB exposure at the exposed and unexposed locations. However, it should be noted that, as in any field study, reference sites are not true controls, and thus, only the absence or presence of parameter disparity can be identified, while causality can only be inferred. Cross-examination of results with "species norms" is utilized here to strengthen conclusions.

Studies of reproductive success were co-located with studies evaluating the concentration of total PCBs and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TEQs), based on World Health Organization (WHO) toxic equivalence factors for birds ($TEF_{WHO-Bird}$) (van den Berg et al., 1998) in the tissues of tree swallows to assess the risk of reproductive effects based on field- and laboratory-derived toxicity reference values (Table 5). The concentrations of total PCBs in the tissues of tree swallows from TB were comparable to concentrations in eggs ($4.1 \mu\text{g PCB/g}$) and nestlings ($3.0 \mu\text{g PCB/g}$) at the Fox River, WI (Custer et al., 1998), where no reproductive abnormalities were reported, but concentrations at TB were 20-fold less than concentrations reported at the

TABLE 5. Mean (SD) Concentrations of Total PCBs and 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin Equivalents (TEQs)^a in the Tissues of Tree Swallows at the Fort Custer State Recreation Area (FC) and at the Trowbridge Impoundment (TB) (Neigh et al., 2006a)

	Total PCBs ($\mu\text{g PCB/g}$)			TEQs (ng TEQ/g)		
	Egg	Nestling	Adult	Egg	Nestling	Adult
FC	0.81 (0.54)	0.46 (0.51)	1.5 (0.65)	0.056 (0.027)	0.02 (0.013)	0.22 (0.03)
TB	5.1 (4.3) ^b	3.1 (1.6) ^b	8.7 (9.7)	0.76 (0.74) ^b	0.6 (0.25) ^b	2.2 (1.8)

^aTEQs based on World Health Organization toxic equivalence factors (van den Berg et al., 1998).

^bMean is significantly greater than at the FC reference area (Student's *t*-test, $p < 0.05$).

Housatonic River, MA (Custer et al., 2003), and the Hudson River, NY (Secord et al., 1999), where reproductive effects potentially exist. TEQ values for tissues at TB were similar to those at other contaminated sites in the Midwest, but the values were calculated with different toxic equivalence factors (Jones et al., 1993; Froese et al., 1998). In addition to PCBs, eggs were found to contain mean (SD) concentrations of 1.5 (1.2) $\mu\text{g DDT/g}$ at TB and 0.45 (0.13) $\mu\text{g DDT/g}$ at FC, but the concentrations were not significantly different between locations. Abandoned tree swallow eggs from another location contained 2.8 $\mu\text{g DDT/g}$, while attended eggs contained 1.3 $\mu\text{g DDT/g}$ (DeWesse et al., 1985). The lack of reproductive effects normally attributed to DDT contamination suggest that although concentrations of DDE may be near a threshold for effects in some bird species (Elliot & Harris, 2002), DDT may not have a significant effect on tree swallows at the Kalamazoo River.

Reproductive Success

Fewer clutches were expected at TB than FC because FC has had a nesting population established for 30 yr with adults and offspring returning to nest annually (Kuerzi, 1941; Cohen et al., 1989; Robertson et al., 1992). The number of nests established by adults increased at TB as the study progressed. It was not possible to determine the proportion of returning birds in the population because nestlings were not banded during the study. Age of females has been identified as an important factor in tree swallow reproduction. SY birds may have lesser reproductive output due to lack of breeding experience (Wheelwright & Schultz, 1994; Saether, 2003) or alterations in spermatogona (Bishop et al., 1998b). These factors may contribute to smaller clutches and lesser productivity in SY birds during portions of the nesting season (De Steven, 1978; Wheelwright & Schultz, 1994; Stutchbury & Robertson, 1988). Although there was a statistically significant difference in female age between years and sites, it is unlikely that reproductive success was affected by the age of the females, since all reproductive parameters were evaluated for covariance with female age and no interaction was significant. This suggests that female age had little effect on the outcome of reproduction at sites along the Kalamazoo River.

Interaction between nestlings could also influence measures of reproductive success such as fledging (Michaud & Leonard, 2000), so reproductive measures were evaluated for interaction with the number of nestlings in the nest at hatch. There was little correlation between the number of nestlings and fledging success ($R^2 = -0.005$), but there was a correlation between the number of nestlings in the nest before a nestling was sampled and productivity ($R^2 = 0.55$), with the productivity increasing with the number of nestlings.

Date of clutch initiation was also considered as a possible cofactor with reproductive success of tree swallows (Stutchbury & Robertson, 1987, 1988; Winkler et al., 2002). Adults who initiate earlier tend to have larger clutches than adults who initiate later in the season, which translates into larger broods and more fledglings from early nesting adults (Stutchbury & Robertson, 1988).

This relationship may not be due to direct interaction between date of initiation and clutch size, but may be due to other confounding factors (Winkler et al., 2002). Nest initiation may be constrained early in the season due to low temperatures (Kuerzi, 1941; Stevenson & Bryant, 2000; Winkler et al., 2002), but adults who nest early may obtain prime nesting locations before other adults arrive at the site (Stutchbury & Robertson, 1988). Adults that nested earlier may also have benefited from coincidental timing of temperature-dependent insect emergence during the nestling period (Stevenson & Bryant, 2000). Timely nesting is beneficial to the growth and development of the nestlings and overall reproductive success because nesting coincides with insect prey abundance during the peak growing period of the nestlings (Quinney et al., 1986). In our studies, there was no interaction between overall productivity and date of initiation, but clutch size did significantly interact with date of initiation in 2000 and 2001. The lack of significant interactions between reproduction and date of nest initiation also suggests that initiation date does not have a significant effect on reproductive outcome at the Kalamazoo River.

Reproductive success, reported as hatching success, fledging success, and overall productivity, are ecologically important parameters that if affected by exposure to PCBs could result in population-level effects. These measures of reproduction were similar between sites and within the normal range for populations as summarized by Robertson et al. (1992). This suggests that the KRAOC population was not exposed to concentrations of PCBs that were sufficient to produce adverse effects. Although it is possible, depending on the viability of the egg sampled for tissue analysis, that hatching rates and brood size could differ from the true value for the nest. The method for assessing reproductive success in this study assumes that the hatching success of the remaining eggs is equivalent to the hatching success of all the eggs before sampling. If hatching success is in fact different, depending on the egg sampled, the hatching success, brood size, and productivity could vary by approximately 0 to 25%. Sampling of eggs was conducted randomly, so it is assumed that over the entire population, the reproductive measurement would reflect a true mean value for the entire population. In addition, this study was designed as a comparative study between an exposed and unexposed population. The sampling of eggs was conducted at both locations, so the effect of this methodology is expected to be similar at both locations. Potentially, the reproductive measurements we report are not comparative to other populations, but they are comparative between the exposed and unexposed population. Even with this issue, reproductive measurements generally fell within the range reported for other populations.

The power to detect differences in the mean between locations was generally insufficient ($1 - \beta < 0.20$), and the power of the analyses was generally insufficient to detect a 20% reduction in reproductive health at TB compared to FC (Table 6). The lesser power is likely due to the variability associated with measures of reproductive outcome, but small sample sizes also contribute to the inability to detect differences. Although power may have been low for

TABLE 6. Power ($1 - \beta$) of Statistical Comparisons Presented in the Text and Sample Size (n) Needed to Detect a 20% Reduction in Reproductive Success at the Trowbridge Impoundment Compared to the Fort Custer State Recreation Area

	2000		2001		2002		All years combined	
	$1 - \beta$	n	$1 - \beta$	n	$1 - \beta$	n	$1 - \beta$	n
Clutch size	0.69	20	0.05	8	0.40	10	0.76	13
Hatching success	0.09	62	0.11	28	0.09	61	0.09	48
Fledging success	NA ^a	NA ^a	0.15	8	0.17	5	0.15	5
Productivity	0.09	62	0.12	37	0.06	68	0.07	55
Predicted brood size	0.10	33	0.05	27	0.05	46	0.16	32
Predicted number of fledglings	0.10	33	0.05	28	0.05	54	0.10	36

^aNA, not available.

statistical tests comparing mean reproductive measurements between locations, the reproductive successes of both the FC and TB population were generally within the normal range reported for other tree swallow populations (Robertson et al., 1992).

Clutch size was the single reproductive endpoint that exhibited a statistically significant difference between locations in some years. In 2 out of 3 years, the clutch size was greater at the reference location than at TB in the KRAOC. However, clutch size at both the FC and TB locations along the Kalamazoo River were slightly less than those reported for other populations (unweighted grand mean [\pm SD] for 28 populations = 5.40 [0.37]) (Robertson et al., 1992). Other studies where tree swallows have been exposed under field conditions to concentrations of PCBs that were similar to or greater than those at the KRAOC did not exhibit effects on clutch size that could be related to exposure to PCBs (Custer et al., 1998, 2003). At some locations with elevated concentrations of PCBs, such as the Hudson River, a greater incidence of supernormal clutches was observed (McCarty & Secord, 1999), and supernormal clutches also have been documented in gull species in contaminated areas (Conover, 1984). Supernormal clutches were not found at TB, but three clutches at FC had seven eggs each.

During incubation, it is possible that abandonment may increase due to contamination (McCarty & Secord, 1999), but there are other factors that can produce abandonment. There were several incidences of interference by house wrens at both FC and TB and several cases of abandonment (FC $n = 2$, TB $n = 4$). The percentage of total nests abandoned at TB (12%) was greater than at FC (3%). Some adults experienced attacks by house wrens during the incubation stage, and therefore, some or all of the documented cases of abandonment may be attributed to this disruption or to inclement weather. Several cases where adults buried eggs with nest material were observed at both sites. This behavior is believed to be a precursor to abandonment (Secord et al., 1999), but in each case of egg burial at TB, the nest was not abandoned.

Although beyond the scope of this paper, it is possible that the eggs were infertile and subsequently buried by the female to better incubate the remaining fertile eggs. It is suggested that male tree swallows exposed to PCBs have abnormal spermatogona, which results in decreased fertility of eggs (Bishop et al., 1998b). Further studies designed to evaluate PCB-induced infertility and resource partitioning would be needed to further address the causes of egg burial and abandonment.

Eggs exposed to PCBs may have a slightly smaller size and mass, but the relationship is not considered to be strong (Secord et al., 1999). Eggs from the KRAOC, where adults were exposed to greater concentrations of PCBs, were significantly heavier, but PCB concentration was not correlated with egg weight or hatchability. Egg weight and length from TB were within the range for unexposed populations, but egg mass at FC was slightly less than for normal populations (De Steven, 1978; Wheelwright & Schultz, 1994). In Western bluebirds (*Sialia mexicana*), exposure to PCBs has been associated with smaller egg volume, which was mainly associated with variation in egg width (Fair & Myers, 2002). In contrast, PCB exposure in one study was linked to larger yolk mass in American kestrels (*Falco sparverius*) (Fernie et al., 2000), which may account for the significantly heavier eggs at TB.

Growth and Growth Curves

Growth parameters and nestlings' weights measured for both sites were within the normal range for tree swallows (Wheelwright & Schultz, 1994; Harris & Elliott, 2000). Thirteen days after hatching, tree swallows at TB were slightly heavier than those at FC, which may be associated with the heavier eggs at TB (St. Louis & Barlow, 1993). It has been suggested that measurements of growth, based on weight, are suitable indicators of stress and fitness (Zach & Mayoh, 1982), but studies measuring the weight of nestlings have not found a causal link between growth in tree swallows and PCB exposure (McCarty & Secord, 1999; Harris & Elliott, 2000). Growth measurements were intended to be integrative measures of behavior, stress, efficiency of catabolism, and feeding behavior. Under a stressor such as exposure to PCBs, the energetics of animals change and growth is expected to decrease (Powell et al., 1996). PCBs have been reported to cause a number of biochemical changes, such as a decrease in vitamin A levels required for growth (Martinovic et al., 2003a). Measures of biomarkers of possible subtle biochemical changes were not measured in this study. Instead, we measured more directly ecologically relevant integrative measures of survival, growth, and reproduction, which are more predictive of population-level phenomena. The greater utility of responses at the biochemical level of organization is used to elucidate possible causes of observed effects on the more ecologically relevant, integrating endpoints.

Growth rates of nestlings were within the range expected for uncontaminated populations (Kuerzi, 1941; Harris & Elliott, 2000). Growth curves for tree swallows on the Kalamazoo River were based on a logistic equation as

suggested by Zach and Mayoh (1982). In our study, the logistic model seems to be an adequate fit over the portion of the nestling period that was evaluated. Mass gain per day, which described the slope of the growth curve at individual boxes, was also not significantly different between sites. This result suggests that there were no PCB-related effects on growth of tree swallow populations within the KRAOC. If there were subtle effects on specific biochemical pathways or substrates, it appears they were well within the normal scope for growth of tree swallows and the organisms easily compensated for any such effects, so there were no adverse effect on overall energetics and growth.

Parental Attentiveness

Some studies have found that behavior of birds can be altered by exposure to contaminants, including PCBs (Peakall & Peakall, 1973; More et al., 1993; Halbrook et al., 1998). These effects are plausible since PCBs are known to cause neurobehavioral effects through specific molecular pathways (Giesy & Kannan, 1998). In doves (*Columba livia*), there was a decrease in nest attentiveness (Peakall & Peakall, 1973), prolonged courtship, and failure to nest when exposed to PCBs (Tori & Peterle, 1983). Likewise, Forster's terns (*Sterna forsteri*) exhibited longer incubation time, less attentiveness to nests, nest abandonment, and egg disappearance (Kubiak et al., 1989). Such an effect on attentiveness of the adults, measured as the number of visits per nestling, was not observed among TB birds in 2000 or 2001, but in 2002, the number of visits was significantly less at TB compared to FC. The difference between sites can not be attributed to environmental factors such as weather or time of sampling because observations were made during several time periods, and there was no change in the significance of the relationship when time of observation and age of nestlings were considered as interacting cofactors. There is some evidence that feeding rates can be increased or decreased during certain periods (Kuerzi, 1941), and it is possible that observations were conducted during slow periods in 2002.

One other possible explanation for fewer feeding visits per nestling at TB during 2002 was the likely relationship between large brood size and the ability of adults to capture prey for the large brood. As brood size increases, the number of feedings per nestling decreases (Leffelaar & Robertson, 1986). The correlation between visits and the number of nestlings was examined, and there was only a slight relationship ($R^2 < 0.20$). This relationship could not account for the observed difference in visitations because brood size at TB during 2002 was smaller than in other years. Tree swallow populations are believed to respond to insect abundance (Quinney et al., 1986), so another explanation for the fewer visits at TB than FC involves the availability of insects at TB during 2002. While the reason for fewer visits observed at TB in 2002 is unknown, the fact that such an effect was not observed in every year suggests that it was not related solely to exposure to PCBs. While it can not be ruled out that this effect might have been produced by some interaction between exposure to PCBs and nutritional or climatic factors, this seems unlikely. Regardless

of the reason, the fewer number of nest visits did not result in differences in growth, survival, or overall productivity.

Gross Morphology and Abnormalities

Tree swallows are relatively insensitive to gross morphological defects produced by exposure to PCBs (McCarty & Secord, 1999) at concentrations greater than the threshold for effects in other species (Barron et al., 1995). However, histological studies have observed changes in spleen and bone tissue of tree swallows (Bishop et al., 1998a). Caspian terns (*Sterna caspia*) in Saginaw Bay exhibited a range of abnormalities, including, among others, scoliosis, clubbed feet, and gastrochisis with body concentrations of 8.0 to 18 µg PCB/g (Ludwig et al., 1993), whereas tree swallows at the Hudson River had concentrations up to 62 µg PCB/g wet weight in nestlings with no morphological abnormalities (Secord et al., 1999). Kalamazoo River populations did not exhibit any morphological abnormalities at concentrations of PCBs as great as 32 µg PCB/g (Neight et al., 2006a).

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