PCB concentrations in tree swallow (*Tachycineta bicolor*) eggs collected from the Upper Hudson River varies within a season and between years.

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Introduction:

Production of polychlorinated biphenyls (PCBs) was banned in the US in 1978, however, their stable nature means they remain chemicals of concern to wildlife and human populations today. Since their ban, PCBs have been determined to be both toxic and carcinogenic to animals at high doses. They have been correlated with adverse health effects including impaired immune, reproductive, nervous, and endocrine function. The Upper Hudson River is considered one of the most PCB-contaminated sites in the country. Eggs from a number of species including tree swallows, spotted sandpipers and belted kingfishers have undergone analysis of PCB concentrations. Mean PCB concentrations in tree swallow eggs were found to be 6800ng/g egg weight in 2004 (Custer et al., 2010b), but values have yet to be considered with respect to annual and within-season variation. Female birds sequester lipid-soluble contaminants such as PCBs in their eggs, so it is possible that a female that produces a larger clutch or more than one clutch may produce eggs with lower PCB concentrations. However, this may be confounded by exposure throughout the season. As insect prey availability increases the PCB body burden may increase to such an extent that PCB concentrations in eggs are actually seen to increase throughout the season.

Methods:

Egg Collection :

Tree swallow (*Tachycineta bicolor*) eggs were collected from nests at the Upper Hudson River and three nesting sites (Patuxent Research Refuge, MD; Upper Sacandaga Lake, NY and Cobleskill Reservoir, NY) with no historical records of PCB usage or spills for comparison. Nest boxes were monitored at regular intervals to determine when nesting behavior and egg laying began.

Egg Contents Removal:

Egg contents were weighed directly into a chemically clean opaque glass jar. Any contents remaining in the egg were transferred with a micropipette. 2.0g of egg contents is required for analysis, so this two eggs were pooled per sample. Wherever possible eggs collected from the same nest were used. Previous research (Custer et al., 2010a) showed that the effect of laying order on PCB concentration in tree swallow eggs varies between years and females, so random egg selection within nests is unlikely to specifically the skew the data.

PCB Analysis:

High resolution mass spectrometry was conducted by Axys Analytical Services (Victoria, BC). Certified reference materials, blanks, duplicates, and spiked samples were were included with each analytical series. Extraction efficiency was monitored using ^{13}C labeled analogs, blanks/spikes, and references. Egg PCB concentrations were back-corrected to fresh wet weight. PCB congeners were analyzed following U.S. EPA Method 1668A (http://www.epa.gov/Region3/1668a.pdf). The median level of detection was <0.5 ng/g wet weight for all congeners except: 0.001 ng/g wet weight for congeners 77, 81,189; <0.005 ng/g wet weight for congeners 157, 167, 169; <0.01 ng/g wet weight for congeners 123, 126, 209; <1.0 ng/g wet weight for congeners 47/48/75, 43/49, 40, 57, 58, 67, 89/90/101, 99, 139/149, 110, 105/127; and <1.5 ng/g wet weight for congeners 66/80, 106/118, 153, 138/163/164, 61/74. Total, dioxin-like and non-dioxin-like PCB concentrations were by summation of PCB congener concentrations. Only concentrations at or above the limit of quantitation were included in the analysis. For analysis of inter- and intra-seasonal variation PCBs were grouped according to being dioxin-like (activate the aryl hydrocarbon receptor) or non-dioxin-like (physiological effects are less well understood) due to possible differences in endpoints for later examination.

Table 1: PCB concentrations measured in tree swallow eggs collected from the Upper Hudson River from 2006 - 2008.

PCB group	2006 (n=22)	2007 (n=32)	2008 (n=43)
	mean + SE (ng/g wet wt egg)		
dioxin-like	193.22 ± 16.14	260.33 ± 40.47	214.43 ±18.75
non-dioxin-like	3336.44 ± 304.00	5656.52 ± 883.69	4651.71 ± 393.02
total	3529.66 ± 318.88	5916.86 ± 923.28	4866.14 ± 411.19

Table 2: PCB concentrations measured in tree swallow eggs collected from 'control' sites from 2006-2008

PCB group	Upper Sacandaga Lake (n=9)	Patuxent (n=7)	Patuxent (n=6)	Cobleskill (n=6)	Cobleskill (n=6)
	2006	2006	2007	2007	2008
	mean + SE (ng/g wet wt egg)				
dioxin-like	26.82 ± 10.06	8.74 ± 1.55	15.64 ± 2.18	10.15 ± 2.10	22.19 ± 4.09
non-dioxin-like	526.92 ± 149.07	273.02 ± 41.00	336.51 ± 36.10	242.31 ± 50.95	714.28 ± 265.14
total	555.23 ± 159.35	282.33 ± 42.46	352.16 ± 38.26	252.46 ± 52.94	736.47 ± 268.14

Figure 1: Dioxin-like PCB content of tree swallow eggs collected at the Upper Hudson River (2006-2008).

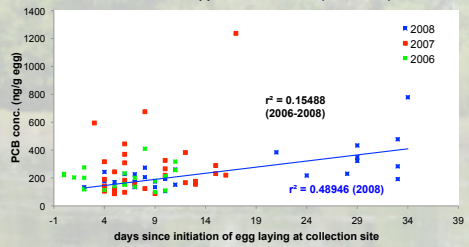


Figure 2: Non-dioxin-like PCB content of tree swallow eggs collected at the Upper Hudson River (2006-2008).

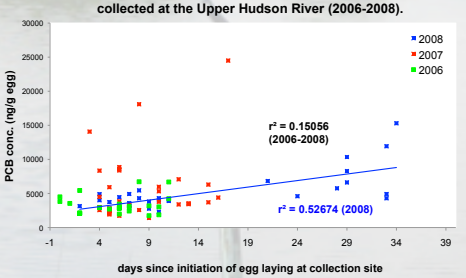
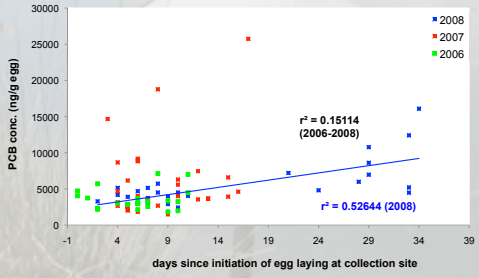


Figure 3: Total PCB content of tree swallow eggs collected at the Upper Hudson River (2006-2008).



Results:

Upper Hudson River vs. Control Sites:

Eggs were collected from three 'control' sites (Table 1) for comparison to PCB content measured at the Upper Hudson River site. In 2006 Upper Sacandaga Lake was chosen as a 'control' site for comparison, however, due to concerns of possible higher than expected PCB contamination of the area it was replaced in 2007 and 2008 with Cobleskill Resevoir. Due to these changes in sampling site it was only possible to compare between sites and within years using t-tests. In 2006 there was no significant difference between the levels of dioxin-like PCBs (df=14; t=1.56; p=0.141) or total PCBs (df=14; t=1.468; p=0.164) between Upper Sacandaga Lake and the Patuxent Research Refuge. Similarily, there were no significant differences in 2007 between levels of dioxin-like PCBs (df=10; t=1.814; t=0.0998) or total PCB (df=10; t=1.526; p=0.158) at Cobleskill Reservoir and Patuxent Research Refuge. There were however, significant differences in dioxin-like PCB levels between 2006 and 2007 at the Patuxent Research Refuge (df=11; t=2.635; p=0.0232) and between 2007 and 2008 at the Cobleskill Reservoir (df=10; t=2.62; p=0.0265). No such differences were detected for total PCB concentrations.

Since there were no differences detected in total PCB concentrations across the 'control' sites these were pooled for comparison to Upper Hudson River eggs. Low sample sizes at the 'control' sites make it impossible to make individual comparisons to PCB egg content of Upper Hudson River eggs.

As expected there were significant differences between both dioxin-like PCB (df=117; t=7.383; p<0.0001) and total PCB (df=117; t=6.875; p<0.0001) content of tree swallow eggs from 'control' sites when compared to egg collected from the Upper Hudson River site. Concentrations of dioxin-like PCBs were 7-30 times greater at the Upper Hudson River site, while total PCB concentrations were 5-24 times higher.

Upper Hudson River - Variation between and within years:

Analysis of covariance showed there was a significant difference in total PCB content (df=2; F=3.285; p=0.04), non-dioxin-like PCB content (df=2; F=3.301; p=0.042) and dioxin-like PCB content (df=2; F=2.941; p=0.059) of tree swallow eggs from the Upper Hudson River between years. There was also a significant difference in total PCB content (df=1; F=4.176; p=0.04) and non-dioxin-like PCBs (df=1; F=6.856; p=0.011), but not dioxin-like PCBs across the number of days since laying was initiated at the site. There were no significant interaction effects between year and days since laying was initiated.

Regression analyses showed that when 'days since laying was initiated' was graphed against PCB concentration, regardless of year, that there was a significant relationship between dioxin-like PCB (Figure 1; p=0.0002), non-dioxin-like PCB (Figure 2; p=0.0002) and total PCB (Figure 3; p=0.0002) concentration. Upon separation of years it was shown this was primarily due to the stronger relationship found in 2008 between PCB concentration and initiation of egg laying (p values = 0.0001 for all three PCB classifications). Time from completion of egg laying to fledging in tree swallows is approximately 30-34 days. Data from 2008 while spread out over this time is unlikely to represent double-clutching of successful nests, but may include some pairs that have lost a clutch to weather or predation.

Discussion:

- As expected, PCB concentrations in tree swallow eggs were significantly higher in eggs collected from the Upper Hudson River compared to 'control' sites
- PCB concentrations at both 'control' sites and the Upper Hudson River can differ between years indicating that environmental factors such as temperature, rainfall and prey availability could influence PCB content of tree swallow eggs.
- PCB content of eggs may also be influenced (at least at the Upper Hudson River site) by timing of egg laying relative to initiation of egg laying at a site.
- Tree swallows that lay eggs later in the season or have a second clutch are likely to be exposed to PCBs for a longer period, allowing for more to be sequestered into eggs prior to laying.
- This effect was primarily seen in 2008 when eggs were collected for a longer time, so further investigation would be required to determine the extent to which this changes on a yearly basis.

Literature Cited:

Custer, C.M., Gray, B.R. and Custer, T.W. (2010a). Effects of egg order on organic and inorganic element concentrations and egg characteristics in tree swallows, Tachycineta bicolor. Environ Toxicol Chem 29(4): 909-21.

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