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ACCUMULATION AND MATERNAL TRANSFER OF POLYCHLORINATED BIPHENYLS IN SNAPPING TURTLES OF THE UPPER HUDSON RIVER, NEW YORK, USA

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Abstract—We conducted field studies over three years to assess body burdens and maternal transfer of polychlorinated biphenyls (PCBs) as well as indices of sexual dimorphism in snapping turtles (*Chelydra serpentina*) of the upper Hudson River (NY, USA.) We collected adult turtles in areas known to be contaminated with PCBs and in nearby reference areas for measurement of body size, precloacal length, and penis size. We analyzed PCB concentrations in eggs collected over three years and in whole blood from adults in one year. Total PCB concentrations (mean ± standard error) in eggs were 2,800 ± 520 and 59 ± 5 ng/g wet weight in the contaminated area and the reference area, respectively. Eggs from the contaminated area were significantly enriched in tri-, penta-, and hepta-PCBs relative to the reference area. Blood from adults in the contaminated area averaged 475 ± 200 and 125 ± 34 ng/g wet weight for males and females, respectively. In the reference area, blood PCB concentrations were 7 ± 3 and 4 ± 1 ng/g wet weight for males and females, respectively. Significant positive relationships were found between carapace length and blood PCB concentration for both sexes in the contaminated area; however, only a marginal relationship was found between female carapace length and concentration of PCBs in their eggs. Our results suggest that PCB contamination of the upper Hudson River presents risks of establishing high body burdens and of maternal transfer of PCBs to eggs, although our measures of gross morphology revealed no discernable expression of abnormal sexual development or reproduction.

Keywords—Reptiles Eggs Persistent contaminants General Electric

INTRODUCTION

From 1947 to 1976, the General Electric Corporation (GE) released polychlorinated biphenyls (PCBs) into the upper Hudson River (NY, USA) from two electric capacitor plants in the communities of Hudson Falls (43°18′N, 73°35′W) and Fort Edward (43°16′N, 73°35′W) (Fig. 1). Approximately 95,000 to 603,000 kg of PCBs were released into the river by the GE facilities before 1977 [1]. The entire 322-km downstream portion of the Hudson River to the New York Harbor was subsequently listed on the Federal Superfund National Priority List in 1984 by the U.S. Environmental Protection Agency. Approximately 80% of PCB inputs into the river consisted of the commercial mixture Aroclor® 1242 (Monsanto, St. Louis, MO, USA), with the remainder comprised of Aroclors 1254, 1221, and 1016 [1,2]. Despite the cessation of PCB use at the GE facilities following federally imposed restrictions, release of PCBs into the river continues from several sources, including erosion of remnant deposits and seepage from bedrock fractures below the plants. Ongoing release from these sources was estimated in 1998 as being approximately 30 kg annually, compared to 2,200 to 16,000 kg/year before 1977 (http://www.seagrant.sunysb.edu/HEP/archive/hrfpcb102901.pdf). Removal of an earthen dam in Fort Edward in 1973, a severe flood event in 1976, and the collapse of a retention structure at the Hudson Falls plant in 1991 led to suspension and downstream transport of large volumes of contaminated sediment, which was largely deposited in the Thompson Island Pool (TIP; ~10 km downstream of Fort Edward). Because of the deposition of highly contaminated sediments in the TIP, this portion of the river remains a major source of PCBs to the water column and to areas downstream [1].

Polychlorinated biphenyl concentrations in the Hudson River generally decrease with distance downstream from the GE plants [2], although significant heterogeneity exists even near source points, as evidenced by the presence of 40 hot spots [1]. In 1991, maximum and average PCB concentrations in surface sediments were 2,000 and 42 mg/kg wet weight, respectively, in the TIP and 4,000 and 26 mg/kg wet weight, respectively, in the 8.2 km downstream of the TIP to the Northumberland Dam [1]. The uppermost section of the Hudson River Superfund Site has largely retained a lower-chlorinated congener pattern, indicative of freshly released Aroclor 1242 from the bedrock below the Hudson Falls plant. Fish species studied in the same area, however, have been characterized as having a congener pattern similar to that of Aroclor 1248 as a result of differential partitioning and accumulation [1].

Numerous studies conducted over the past several decades have addressed PCB accumulation by fish in the upper Hudson River system [1,3] for the purposes of monitoring contaminant trends and assessing human health risks associated with fish consumption. Commonly consumed fish species as far as 56 km downstream (to the Federal Dam, Troy, NY, USA) contain average PCB concentrations of 1.6 to 41 μg/g in edible fillet portions—levels that exceed the risk-based ''do not eat'' concentration of 2 μg/g [1]. In several other studies, birds in the highly contaminated area were monitored for PCB contamination and found to have exceptionally high body burdens and egg concentrations, particularly in insectivorous and piscivorous species [4,5]. Echols et al. [4], for example, found PCB

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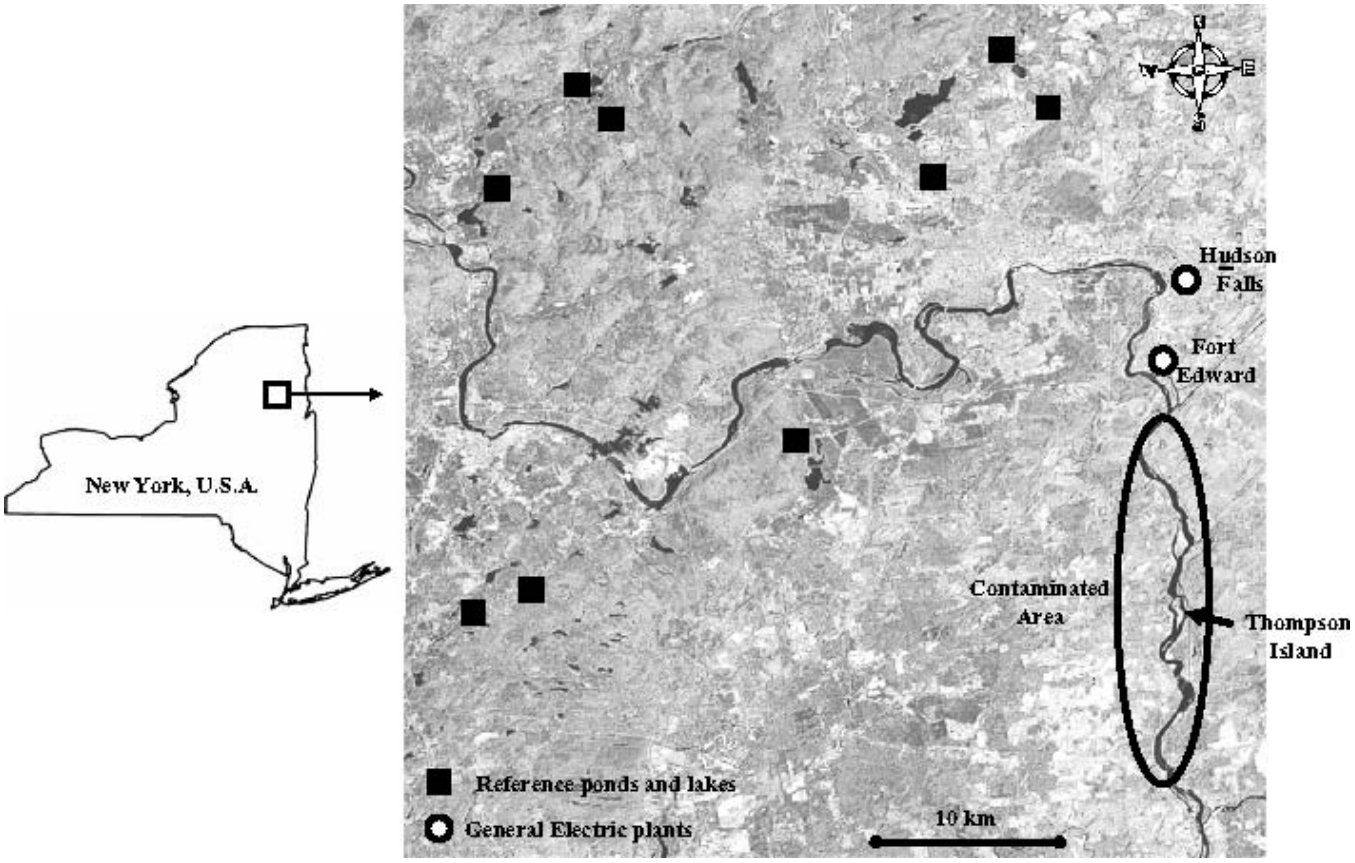

Fig. 1. Locations of the study areas, upper Hudson River (NY, USA).

levels in eggs of insectivorous tree swallows ranging from 13 to 24 μg/g wet weight, whereas adult whole-body PCB concentrations were as high as 152 μg/g wet weight. In addition, predatory aquatic mammals have been monitored and found to possess PCB concentrations in liver as high as 431 μg/g lipid (in otters, a carnivorous species [6]).

The common snapping turtle (*Chelydra serpentina*) has received only limited study despite its wide application as a model species for examining contaminant accumulation and effects in other systems [7,8]. A long-lived species of high trophic position, snapping turtles have the propensity to accumulate high concentrations of lipophilic contaminants and transfer them to their eggs [9,10]. In the 1980s, two small-scale sampling efforts in the upper Hudson River revealed exceptionally high body burdens in both adults and eggs [11,12]. In what is to our knowledge the only ensuing study [13], snapping turtle eggs were found to continue to possess high body burdens of PCBs despite the cessation of active PCB inputs to the river more than two decades earlier. This limited availability of recent data and a dearth of information regarding potential ecological effects of PCBs on snapping turtles in the upper Hudson River prompted us to conduct a comprehensive monitoring effort to assess PCB accumulation, maternal transfer, and potential expression of effects on secondary sexual traits and fitness of resident adults. Over three years, we collected adult snapping turtles from the upper Hudson River region in locations considered to be either highly contaminated or relatively uncontaminated with PCBs. In all years, we collected eggs for assessment of embryonic exposure; however, eggs were not collected from any of the same females from one year to the next. In one year, we also sampled whole blood from adults as a nondestructive index of relative body burdens. Furthermore, comparisons of blood and egg concentrations were used to examine relationships between female body burdens and transfer of PCBs to their eggs. Because of the potential for PCBs to exert estrogenic effects [14], we also measured morphological traits in adult turtles that we hypothesized may reflect alterations in sexual development [8,15,16].

MATERIALS AND METHODS

Study areas

Studies were conducted from 2003 to 2005 in northern New York (USA) (Fig. 1) in areas designated as *reference* or *contaminated* based on their proximity to the GE facilities responsible for PCB releases. The contaminated area consisted of the length of the Hudson River from just south of Fort Edward (location of the southernmost of the two GE plants) to the Northumberland Bridge. This 20-km reach of the river encompasses 35 of the 40 most-contaminated hot spots, defined as having sediment PCB concentrations exceeding 50 μl/L [1]. Ponds, marshes, and abandoned canals within approximately 350 m of the river proper were also included, because snapping turtles may move throughout waterways within their home territories, which can range from 0.7 to 28 ha [7,17]. The reference area consisted of nine relatively pristine lakes and ponds located north and west of the highly contaminated portion of the river (Fig. 1). In both the contaminated area and the reference area, specific collecting locations varied somewhat from year to year, depending on landowner

permission and previous experience in locating adults and nests.

Field collections

All protocols were executed under the terms of a License to Collect or Possess granted by the New York State Department of Environmental Conservation, Division of Fish and Wildlife. Protocols also were approved by the Institutional Animal Care and Use Committee of the University of Maryland Center for Environmental Science.

Adults. From mid-May through late June of all years, coincident with peak nesting season, we captured snapping turtles on land by daily surveys and in the waterways using baited hoop traps. The location of each capture was recorded using a handheld Global Positioning System unit.

All turtles were measured for wet weight, carapace length (CL), and precloacal length (PCL; the distance from the posterior edge of the plastron to the cloacal opening). The ratio of PCL to CL or to the posterior lobe of the plastron is an indicator of the sex of snapping turtles, and it also may serve as an index of relative genitalia size [8]. For males, length and maximum diameter of the penis were measured as well. Penis measurements followed extension of the penis from the cloaca via suspending the individual in a nearly vertical position; typically, the penis was extended to maximum size within 5 min. In 2004, blood samples were drawn from the caudal vein using a 21-gauge, 10-ml, heparinized syringe and then frozen for subsequent chemical analyses. The volume of blood drawn from an individual varied based on body size and estimated total blood volume (5–8% of total body mass [18]), because removal of up to 10% of total blood volume has been shown to have no adverse health effects [18]. Before release at the site of capture (within 24 h of capture, with the exception of females for which oviposition would be induced; see below), individuals were given unique marks consisting of drill holes in the marginal scutes [19] for identification of recaptured individuals in subsequent years.

Eggs. Eggs were collected either from nests or by chemically induced oviposition in gravid females by intraperitoneal injection of oxytocin (20 IU/kg body wt [20]). Following collection, eggs were cleaned with tap water, labeled with a pencil, weighed, and measured. Three eggs were randomly selected from each clutch and frozen at −20°C for PCB analysis. Remaining eggs were used in subsequent incubation studies (to be reported elsewhere).

Chemical analyses

Chemicals. All solvents used in laboratory procedures were of ultrahigh-purity grade and purchased from J.T. Baker (Phillipsburg, NJ, USA). The PCB surrogate standards, internal standards, and Aroclor mixtures were purchased from AccuStandard (New Haven, CT, USA).

Eggs. Three eggs from each clutch were pooled for analysis, because previous research has found that within-clutch variation in egg contaminant concentration is very low [7]. In preparation for analyses, eggs were thawed and rinsed with distilled water, and the contents were thoroughly homogenized. Five grams of the egg homogenate from each clutch were ground with an excess of anhydrous Na_2SO_4 (EMD Chemicals, Gibbstown, NJ, USA) and then spiked with a solution containing the following PCB surrogate standards: 3,5-Dichlorobiphenyl (PCB 14), 2,3,5,6-tetrachlorobiphenyl (PCB 65), and 2,3,4,4′,5,6-hexachlorobiphenyl (PCB 166). Following a 24-h Soxhlet extraction with dichloromethane, samples were concentrated and exchanged into hexane at a volume of approximately 4.0 ml.

A subsample of the extract was used for gravimetric determination of lipid content, and the remaining sample was subjected to lipid removal using gel permeation chromatography with a Phenomenex Phenogel™ column (length, 250 mm; inner diameter, 22.5 mm; 10-μm particles with a 100-Å pore size; Phenomenex, Torrance, CA, USA) using dichloromethane as the mobile phase at a flow rate of 5 ml/min. Samples were further cleaned and the PCB fraction separated from other organic components using 8 g of 2.5% deactivated Florisil® (60–100 mesh; J.T. Baker) in a glass column eluted with 35 ml of 1:1 dichloromethane:petroleum ether (J.T. Baker). The cleaned extracts were concentrated to 1 ml for analysis.

Blood. The liquid–liquid extraction method for turtle whole blood was adapted from a method for measuring organochlorines in sea turtle blood developed by Keller et al. [21]. Blood samples were thawed and spiked with the same PCB surrogate standards used for egg analyses. Because PCBs bind to serum proteins [22], samples were treated with 4.0 ml of 98% formic acid (EMD Chemicals) and sonicated for 15 min to denature the serum proteins and release bound PCBs. Samples were combined with 8.0 ml of 1:1 methyl-*tert*-butyl-ether:hexane, vigorously vortexed for 2 min, and centrifuged for 10 min at 2,000 rpm. The organic layer was transferred to a clean test tube, and the extraction was repeated two more times, with all the organic phases combined. To remove any residual water, the combined organic extracts were mixed with Na_2SO_4. The samples were then concentrated, and lipid content was determined gravimetrically on a subsample of the extract. Lipids and other interfering matter were removed from the remaining sample by passage through a glass column packed with 4 g of 6% deactivated neutral alumina (80–200 mesh; EMD Chemicals) eluted with 35 ml of petroleum ether. The final cleanup step was Florisil column chromatography (described above), followed by sample concentration to 1.0 ml for analysis.

Instrumental analysis of PCBs. The same methods were used to analyze PCBs in final extracts from both eggs and blood. The internal standards 2,3,6-trichlorobiphenyl (PCB 30) and 2,2′,3,4,4′,5,6,6′-octachlorobiphenyl (PCB 204) were added to each sample. The PCBs were quantified using a Hewlett-Packard (Avondale, PA, USA) model 5890 gas chromatograph equipped with a ^{63}Ni electron capture detector, with hydrogen and nitrogen used as carrier gas and makeup gas, respectively. A 5% phenyl-methyl silicone DB-5MS capillary column (length, 60 m; inner diameter, 0.25 mm; film thickness, 0.25 μm) was used with inlet pressure of 100 kPa. The oven-temperature program was as follows: 100°C hold for 2 min, ramp from 100 to 170°C at 4°C/min, ramp from 170 to 280°C at 3°C/min, and a final, 5-min hold at 280°C. The injector temperature was held at 225°C and the detector temperature at 285°C.

Individual PCB congeners were identified based on chromatographic retention times in comparison to those of added internal standards and those of a calibration standard prepared with commercial Aroclor mixtures. Quantification of PCB congeners was performed using the relative response factors generated from the calibration curve of the calibration standard. Congeners quantified in all samples analyzed for PCBs are presented in the *Supporting Information* (http://dx.doi.org/10.1897/08-098.S1); in cases of coeluting congeners, combined values are reported. To classify congeners by homolog

group, coeluting congeners were separated based on the relative contribution in commercial Aroclor mixtures, based on the work of Frame et al. [23]. Several congeners were excluded from all analyses because of loss attributable to high volatility (congeners 1 and 3) or analytical interference from coeluting compounds of unknown identity (congeners 49, 97, and 199).

Quality assurance. Laboratory blanks were analyzed concomitantly with samples. For the egg samples, laboratory blanks consisted of 50 g of sodium sulfate spiked with the PCB surrogate solution and then extracted and processed alongside the samples in an identical manner. Laboratory blanks for the whole-blood samples were comprised of 4.0 ml of ultrapurified water combined with 4.0 ml of formic acid spiked with surrogate solution. The detection limit for each peak, whether a single congener or a group of coeluting congeners, was determined by multiplying the mean mass measured in the blanks by a factor of three. Total PCB (t-PCB) detection limits ranged from 7.2 to 25.7 ng for the egg analyses and from 8.4 to 22.9 ng for the blood analyses. The method detection limit was calculated for each congener in the samples on a concentration basis by dividing the detection limit mass by the mass of sample extracted. The t-PCB concentrations were calculated as the sum of the concentrations of the individual congeners.

For the egg samples, the recoveries (mean ± standard deviation) of surrogate congeners were 84.7% ± 18.9% for PCB 14, 84.0% ± 9.0% for PCB 65, and 89.2% ± 10.6% for PCB 166. For the whole-blood samples, surrogate recoveries were 46.8% ± 22.8% for PCB 14, 62.7% ± 23.9% for PCB 65, and 76.5% ± 22.5% for PCB 166. No systematic differences in recoveries were found between reference and contaminated areas, and reported PCB concentrations for all samples were not corrected for surrogate recoveries. The standard reference material analyzed in conjunction with the snapping turtle egg samples was ''Lake Superior Fish Tissue'' (1946), prepared by the National Institute of Standards and Technology (NIST). Recovery of PCBs measured in this material was 91.6% ± 22% for all certified values. The standard reference material analyzed with the snapping turtle whole blood was ''PCBs, Pesticides, and Dioxins/Furans in Human Serum'' (1589a), also prepared by the NIST. Analysis of this material resulted in PCB concentrations averaging 110% ± 22% for all certified values.

Statistical analyses

Statistical analyses were conducted using Minitab® software (Ver 13; Minitab, State College, PA, USA) with the exception of post hoc power analyses, which were conducted using Systat® software (Ver 11; Systat, San Jose, CA, USA). Data for endpoints measured in multiple years were combined over all years before analysis. Data were initially tested for normality and homoscedasticity to verify that assumptions for parametric analyses were met. Variation in t-PCB concentrations was greater in tissues from the contaminated area than in those from the reference area, and they were subjected to rank transformation [24] before comparisons among sites by analysis of variance (ANOVA). Relative contributions of specific homolog groups to the t-PCB concentrations did not require transformation and were analyzed by multivariate ANOVA followed by univariate comparisons. Data regarding size metrics for genitalia versus t-PCB concentration in blood within study areas were $\log_{10}$-transformed to normalize distributions before analysis by ANOVA or linear regression. Analysis of variance was used to compare morphometric data among study areas. Because of the small number of paired samples of blood and eggs obtained to assess maternal transfer of PCBs to eggs (2004 only, $n = 9$; most eggs were derived from nests rather than from captured females), data were analyzed using linear regression ($\log_{10}$-transformed) based on both areas combined in addition to within each study area. Because different tissue types were being compared when examining maternal transfer of PCBs (blood and egg contents), analyses were conducted based on lipid-normalized concentrations as well as on concentrations per unit wet weight [25]. Comparisons of egg to blood ratios of specific congeners versus log octanol–water partition coefficient (K_{OW}) and of egg PCB concentrations versus clutch size or egg size were conducted using linear regression. Results are presented as means ± standard error. An a priori Type I error rate of 0.05 was used for all statistical tests. However, we note that our statistical power to detect significant differences at the 0.05 level was quite low for some tests (discussed below when relevant to interpretation of results); thus, caution should be used in assessing significance based on this arbitrary Type I error rate, which may be quite conservative when applied to these data [26].

RESULTS

In 2005, 23 and 28% of adults captured in the reference area and the contaminated area, respectively, had been captured and marked in the previous two years, reflecting our repeated use of the same collection sites, the relatively small home range of adults, and fidelity to nesting sites displayed by females. We were not able to recapture any gravid or nesting females between study years; thus, egg data were combined over years without concern for repeated measures. The t-PCB concentrations in eggs were significantly greater in the contaminated area relative to the reference area (Table 1), differing by two orders of magnitude on average. Homolog profile patterns in eggs differed among areas; eggs from the contaminated area were significantly enriched in tri- and hepta-PCBs and marginally elevated in penta-PCBs compared to eggs from the reference area (Fig. 2).

The t-PCB concentrations in blood (2004 only) were significantly elevated in both males and females collected in the contaminated area compared to those collected from the reference area (Table 1). Although average t-PCB concentrations in male blood appeared to exceed those in female blood in the contaminated area, the differences were not significant ($p = 0.221$), which may have been the result of greater variability among males than among females (Table 1). No indication of a difference in t-PCB concentrations between males and females was observed in the reference area ($p = 0.692$). Homolog profiles of blood samples collected from adults in the contaminated area had a greater proportional contribution of tetra-PCBs (females) and penta-PCBs (males and females) relative to reference samples, whereas reference samples had a greater proportional contribution of hepta-PCBs (males and females) and of nona- and deca-PCBs (females) relative to contaminated samples (Fig. 3).

Despite the small number of paired samples of blood and eggs that were available, significant relationships were found among t-PCB concentration in maternal blood and eggs. The relationship was strongest within the contaminated site, whereas in the reference area, in which the range in concentrations was relatively small, the relationship was not significant (Fig. 4). When the sites were combined and a wide range in con-

Table 1. Total polychlorinated biphenyl (PCB) concentration (ng/g wet wt) and lipid content (%) of eggs (all years combined) and whole blood, 2004)[a]

				Blood					
	Eggs			Females			Males		
Area	*n*	$[PCB]_{Total}$	% Lipid	*n*	$[PCB]_{Total}$	% Lipid	*n*	$[PCB]_{Total}$	% Lipid
Reference	29	59.3 ± 4.9 (3.9–107.4)	7.2 ± 0.2 (5.0–8.5)	29	4.0 ± 0.5 (0.8–14.4)	0.37 ± 0.05 (0.09–1.27)	16	7.1 ± 2.8 (0.2–46.4)	0.32 ± 0.06 (0.11–0.94)
Contaminated	46	2,800.0 ± 517.0 (56.1–12,100.0)	7.2 ± 0.4 (3.0–12.4)	22	125.0 ± 33.7 (2.8–663.0)	0.32 ± 0.04 (0.09–1.03)	15	475.0 ± 198.0 (6.0–2,770)	0.36 ± 0.06 (0.12–0.94)
		$p < 0.001$	$p = 0.892$		$p < 0.001$	$p = 0.414$		$p < 0.001$	$p = 0.667$

[a] Values are presented as the mean ± standard error, with the range in parentheses. Data were subjected to rank transformation before comparisons among sites by analysis of variance. *n* = number of clutches from which a pooled sample of three eggs was analyzed.

centrations was captured in our analyses, very strong relationships were found between t-PCB concentrations in maternal blood and eggs, both on a wet-weight and a lipid-normalized basis (wet wt: $r^2 = 0.970$, $p < 0.001$; lipid normalized: $r^2 = 0.952$. $p < 0.001$). No relationship between the egg to blood ratio of specific congeners and the K_{OW} of the congeners was found in either site, but a positive trend was observed at the reference site (Fig. 5).

Comparison of female body size (CL) versus egg t-PCB concentration also suggested a linear relationship (Fig. 6) in the contaminated site, albeit not being statistically significant at the 0.05 level ($p = 0.087$). For these data, however, our statistical power to detect correlations that significantly differed from zero was only 0.40; thus, our test of significance likely was very conservative. Total PCB in blood also was related linearly to CL in both females and males in the contaminated area, although CL only explained a small proportion of the total variance in t-PCB concentrations ($r^2 = 0.391$ and 0.308, respectively). No relationship between CL and blood t-PCB concentrations was found in either sex in the reference area.

Although we observed no linear relationship between egg size (wet wt or diameter) and t-PCB concentration in eggs in either site ($p = 0.067$–0.230), we found overall, site-specific differences in average egg weight per clutch. Average egg wet weight over all years was larger in the reference area than in the contaminated area (11 ± 0.35 vs 10 ± 0.16 g, respectively; $p = 0.004$), whereas egg diameters did not differ ($p = 0.825$) among sites. Despite the lack of a linear relationship between female CL and clutch size in the reference area ($r^2 = 0.027$, $p = 0.649$), these variables were positively related in the contaminated area ($r^2 = 0.355$, $p = 0.015$) At both sites, the t-PCB concentration in eggs was positively related to clutch size (reference: $r^2 = 0.275$, $p = 0.021$; contaminated: $r^2 =$

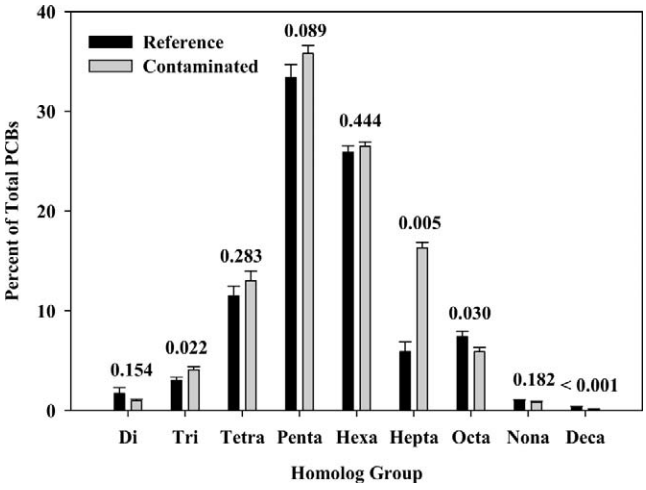

Fig. 2. Relative contributions of homolog groups to total polychlorinated biphenyl (PCB) concentrations in eggs over three years. Values are presented as the mean ± standard error. The *p* values from intrahomolog comparisons are above the bars.

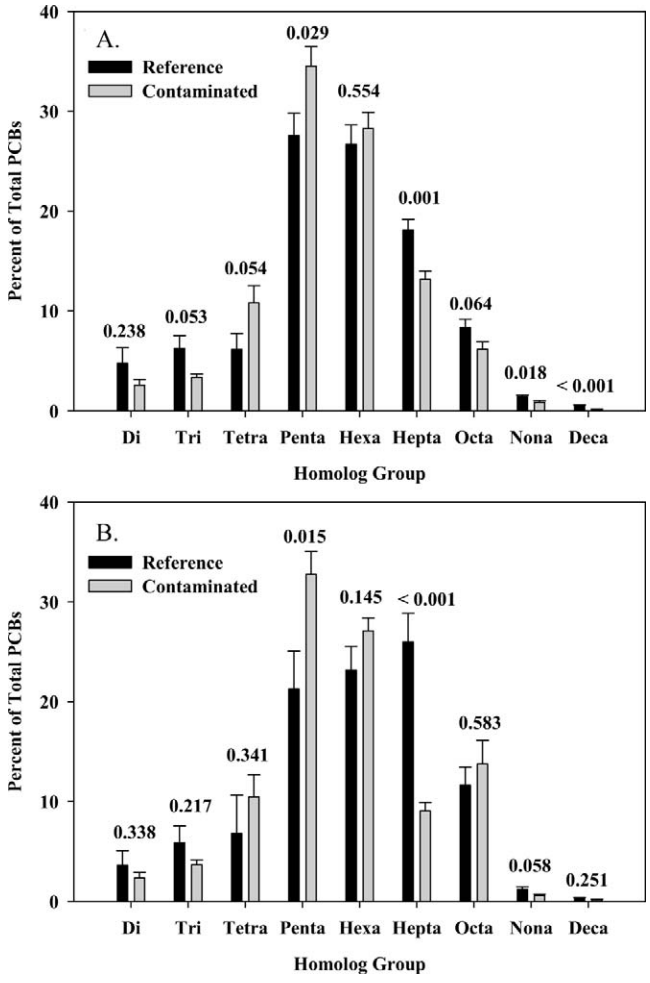

Fig. 3. Relative contributions of homolog groups to total polychlorinated biphenyl (PCB) concentrations in whole blood collected from females (**A**) and males (**B**) in 2004. Values are presented as the mean ± standard error. The *p* values from intrahomolog comparisons are above the bars; the *p* values represent the results of univariate comparisons following multivariate analysis of variance.

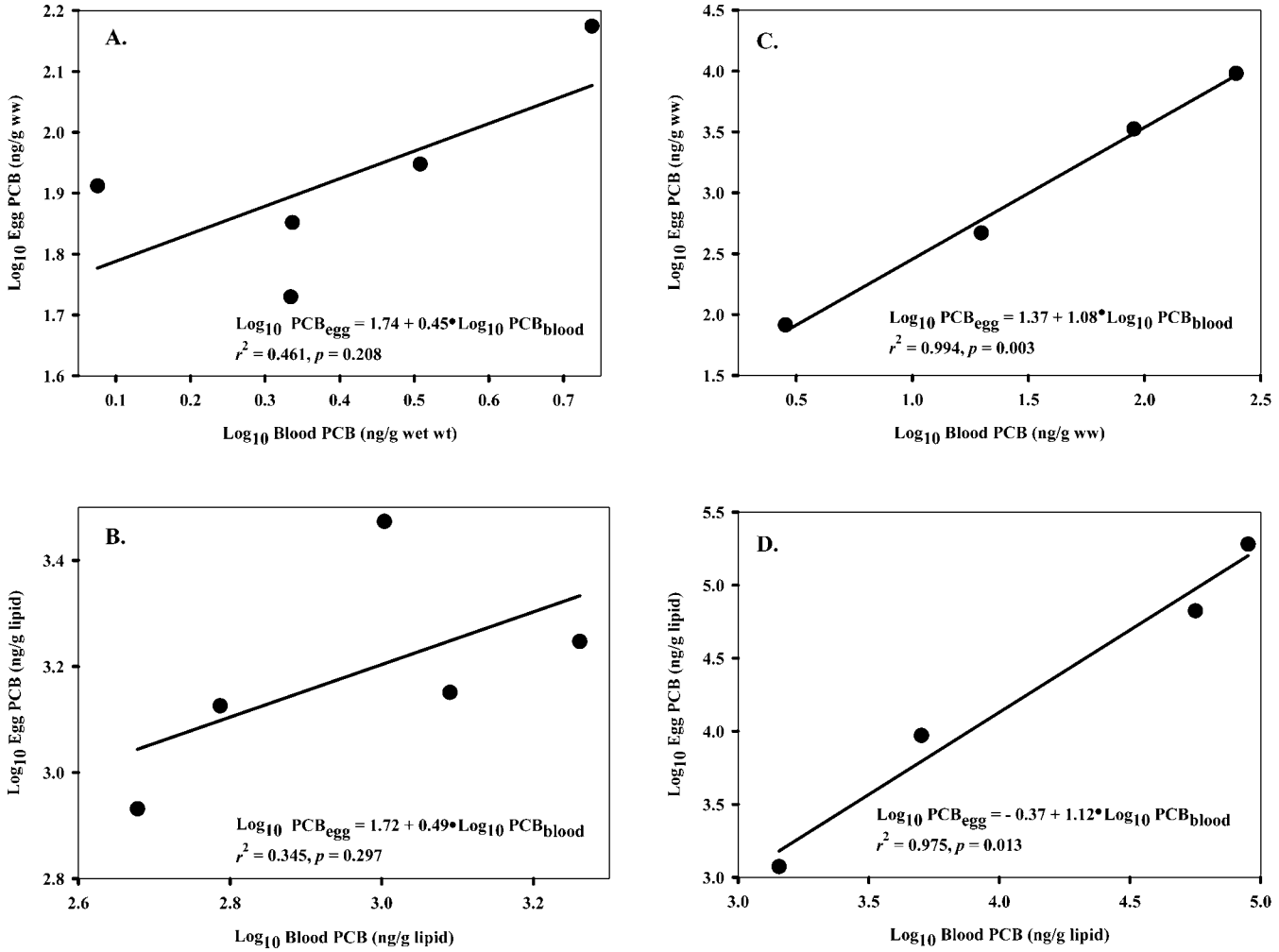

Fig. 4. Relationships between total polychlorinated biphenyl (PCB) concentrations in whole blood and eggs, 2004: (**A** and **B**) reference area; (**C** and **D**) contaminated area. Data are presented on both a wet-weight and a lipid-normalized basis.

0.450, $p = 0.006$). We observed no statistical differences among study areas in adult size or metrics of sexual development (Tables 2 and 3). Regression analysis revealed no relationships between t-PCB concentrations in blood and either PCL to CL ratios or penis length to CL ratios within sampling areas ($p > 0.114$ for all comparisons).

DISCUSSION

The average concentration of t-PCBs in snapping turtle eggs collected from the contaminated site was 47-fold higher than the average in the reference site. The concentrations that we observed in both the contaminated and reference areas were somewhat lower than those observed in previous studies conducted in the same general areas. Analysis of six clutches from the upper Hudson River in 1978 (one year after PCB production was banned in the United States) revealed t-PCB concentrations of 10,400 to 42,900 ng/g wet weight (mean, 28,900 ng/g wet wt) [12], compared to our values in the contaminated site of 56 to 12,100 ng/g wet weight (mean, 2,800 ng/g wet wt). In more recent sampling in 2002, eggs from 11 clutches collected in sites encompassing much of the contaminated area that we sampled revealed t-PCB concentrations of 70 to 31,800 ng/g wet weight (mean, 9,840 ng/g wet wt) [13].

The wide range of t-PCB concentrations measured in snapping turtle eggs collected from the contaminated upper Hudson River area suggests that considerable variability in accumulation and maternal transfer exists within the region, particularly when the most recently published study [13] is compared with ours. Because the upper Hudson River is characterized by localized hot spots of sediment contamination, proximity of the home range to highly contaminated sites and the feeding preferences of any given female likely have strong influences on body burdens and transfer. Note that we observed similar concentrations in the reference area (4–107 ng/g wet wt) to those reported in the 2002 study (two reference areas, ranges of 32–565 and 10–57 ng/g wet wt [13]), suggesting much lower spatial variation in concentrations in the reference area, as would be expected considering that atmospheric deposition likely would account for the reference-area PCB concentrations. It is not possible to determine the absolute range in concentrations in eggs from the entire population, but our results are based on a much larger number of clutches in the contaminated area compared to that in the 2002 study ($n = 46$ vs $n = 11$, respectively [13]) and, thus, are more likely to approach the actual range of concentrations present in eggs of the local population.

Spatial variation in environmental concentrations of PCBs may explain some of the discrepancies in results among studies conducted on the upper Hudson River, but temporal trends also play a role. The highest concentrations of PCBs in eggs from

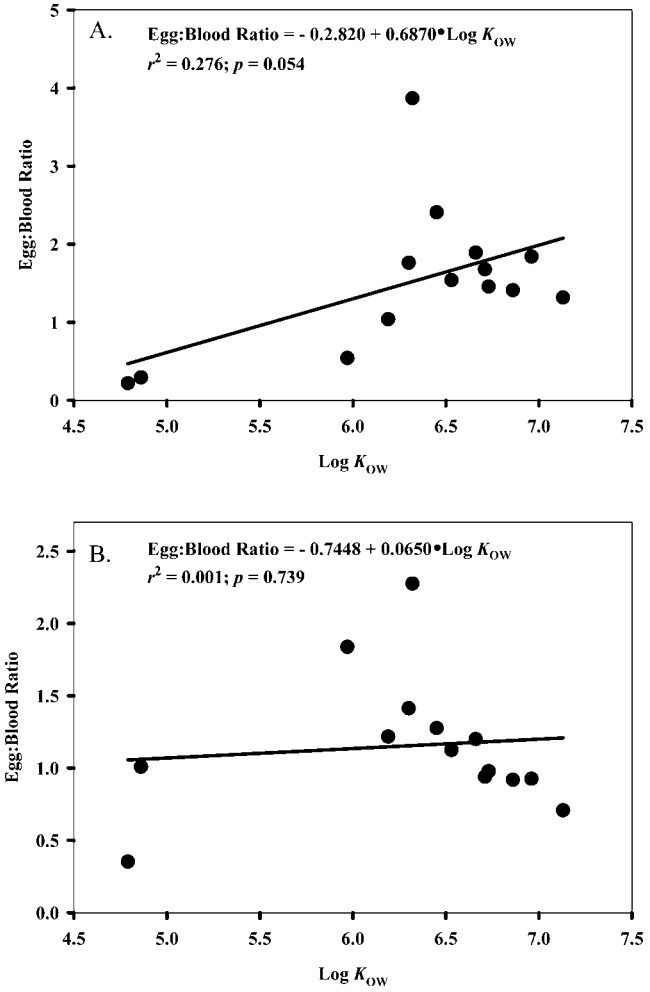

Fig. 5. Congener-specific ratios of polychlorinated biphenyl (PCB) concentrations in eggs and blood from the female producing the eggs (2004) versus log octanol–water partition coefficient (log K_{OW}) of the congeners: (**A**) reference area; (**B**) contaminated area.

the contaminated region were reported in eggs collected in 1978 [12], one year following cessation of PCB production by the local GE plants. Because our data are based on samples collected 26 to 28 years after PCB production had ceased, exposure of females to PCBs is likely to have declined during the time between these studies. Inputs to the Hudson River continue because of leakage from the GE facilities and sediment hotspots (particularly TIP), but these inputs are much lower than they were during active production (http://www.seagrant.sunysb.edu/HEP/archive/hrfpcb102901.pdf).

Reports of high PCB concentrations in snapping turtle eggs are not restricted to populations in the Hudson River. Most notably, studies conducted in Canadian portions of the Great

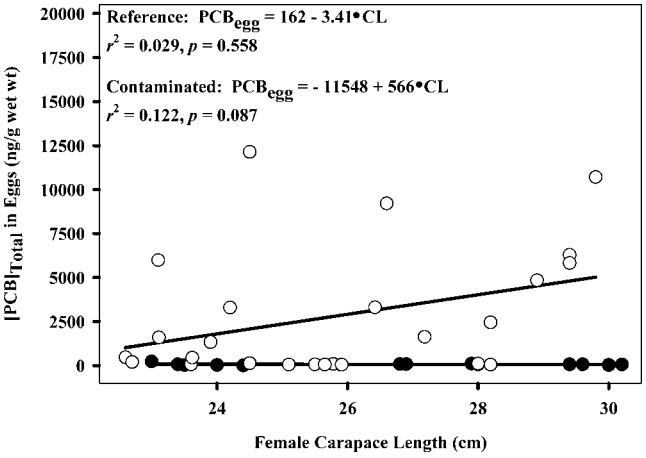

Fig. 6. Relationships between body size (carapace length [CL]) of females and total polychlorinated biphenyl (PCB) concentrations in their eggs collected in the reference area (solid symbols, lower line) and the contaminated area (open symbols; upper line) over three years. Equations are derived from least-squares regression.

Lakes and the St. Lawrence River Basin have found PCB concentrations in eggs that are similar to those we observed or, in some cases, exceed them [9,27–30]. In two instances, eggs were found to have exceptionally high t-PCB concentrations that far exceeded those observed in the upper Hudson River; Ashpole et al. [30] detected 61,000 ng/g wet weight of t-PCBs (1,034,000 ng/g lipid) in a clutch of eggs collected in 1999 from a contaminated site on the south side of the St. Lawrence River. At the same site, a clutch sampled in the previous year was found to have an astonishingly high PCB concentration of 737,700 ng/g wet weight (5,083,000 ng/g lipid)—almost 1% the wet mass of the egg [29]. These values were much greater than the values we obtained (12,100 ng/g wet wt or 127,000 ng/g lipid), but they were restricted to a single waterway with, perhaps relatedly, a very sparse snapping turtle population. Otherwise, average t-PCB concentrations in nearby Canadian Great Lakes–St. Lawrence River Basin areas of concern (AOCs) generally ranged from 340 to 11,000 ng/g wet weight [27–30], similar to those that we observed in the contaminated area of the upper Hudson River. The reported t-PCB concentrations in eggs from reference areas sampled during the studies of the Great Lakes–St. Lawrence River Basin ranged from 15.7 to 272 ng/g wet weight [9,28–30], similar to the concentrations we observed in reference areas.

Two studies elsewhere in North America also reported t-PCB concentrations in snapping turtle eggs similar to those observed by us and others. Pagano et al. [10] reported t-PCB concentrations in snapping turtle eggs from six clutches collected in western and central New York of 1,100 to 310,000

Table 2. Sizes of adults captured in all years in reference and contaminated areas[a]

	Males				Females			
Site	*n*	Mass (kg)	CL (cm)	PCL:CL	*n*	Mass (kg)	CL (cm)	PCL:CL
Reference	50	7.7 ± 0.5	30.6 ± 0.9	0.39 ± 0.01	80	4.2 ± 0.1	26.3 ± 0.3	0.25 ± 0.005
Contaminated	43	7.4 ± 0.6	29.8 ± 1.0	0.38 ± 0.01	78	3.4 ± 0.3	25.7 ± 0.3	0.25 ± 0.004
		p = 0.387	p = 0.158	p = 0.702		p = 0.312	p = 0.091	p = 0.540

[a] Values are presented as the mean ± standard error. Data were compared among sites by analysis of variance. CL = carapace length; PCL = precloacal length.

Table 3. Penis size to body size ratios for adults captured in reference and contaminated areas during all years[a]

Site	*n*	Penis length:body mass	Penis length:CL	Maximum penis diameter:body mass	Maximum penis diameter:CL
Reference	42	0.73 ± 0.11	0.17 ± 0.005	0.49 ± 0.03	0.12 ± 0.003
Contaminated	37	0.82 ± 0.06	0.16 ± 0.006	0.57 ± 0.05	0.11 ± 0.004
		$p = 0.335$	$p = 0.125$	$p = 0.158$	$p = 0.360$

[a] Data were $\log_{10}$-transformed to normalize distributions before analysis by analysis of variance. CL = carapace length.

ng/g lipid, a range encompassing the combined lipid-normalized data from our study areas. In a recent study of t-PCB concentrations in snapping turtle eggs collected from four AOCs in the Lake Erie region of Ohio (USA), concentrations ranged from 873 to 3,680 ng/g wet weight [31], falling within the range of values that we observed. The reported mean t-PCB concentrations at two reference sites in the present study were 183 and 352 ng/g wet weight, exceeding those in our reference area. In all the studies cited above, large intrasite variation was observed in t-PCB concentrations, consistent with our results. This variation was attributed to differential feeding preferences, home range areas, and metabolic activity [7,9,10,27–29], which, if also occurring in the populations that we studied, would be expected to exacerbate the variability we suspect was related to spatial heterogeneity in PCB concentrations in sediments of the upper Hudson River.

Analysis of whole blood for t-PCBs also showed elevated concentrations in adults captured in the contaminated area relative to the reference area. Few other studies have measured PCBs in snapping turtle blood, and those studies typically measured concentrations in plasma rather than whole blood [8,31]. Our results, however, should be comparable with those based on plasma, because Keller et al. [21] concluded that although 81 to 95% of PCBs partition into the plasma component of blood, whole-blood analyses reflect the same concentrations. Concentrations of PCBs in plasma of male snapping turtles from contaminated areas in the Great Lakes AOCs in Ontario (Canada) had mean t-PCB concentrations of 263 to 415 ng/g wet weight [8]. We observed similar average concentrations in whole blood from males in the contaminated area (450 ng/g wet wt), yet the highest concentration (2,650 ng/g wet wt) was nearly sixfold greater than the highest average value reported in Ontario [8]. Plasma PCB concentrations in snapping turtles inhabiting Great Lakes AOCs in Ohio (average, 74–201 ng/g wet wt) also were lower than our observations for whole blood in animals from the contaminated area [31]. Plasma PCB concentrations in reference areas in these studies [8,31] in the Great Lakes (average, 18–27 ng/g wet wt) exceeded those that we measured in the reference area of our study (4–7 ng/g wet wt for females and males), as did PCB concentrations in eggs from reference locations in the Ohio AOC [31] in comparison with our findings. These differences likely reflect a greater degree of background contamination in the Great Lakes region than in upstate New York because of more widespread and heavy industrialization.

The coeluting congeners 132, 153, and 105 were the dominant congeners measured in egg and blood samples from contaminated and reference areas in the present study. These congeners comprised 15 to 28% of the t-PCB concentrations. Whereas PCB 105 would have been the highest of the three congeners comprising this peak in the dominant Aroclor originally released to the river (Aroclor 1242), it is relatively rapidly metabolized by cytochrome P450 enzymes [32] such that the vast majority of this peak in our samples was PCB 153, a recalcitrant congener that is nearly ubiquitous in environmental samples [33]. The other dominant congeners in our samples were PCBs 138, 180, and 118, all of which are considered to be highly recalcitrant in environmental samples [33].

Given that the sediments in the highly contaminated region of the upper Hudson River are characterized by the dominance of lighter congeners (Aroclor 1242), it is somewhat surprising that egg and whole-blood samples did not have proportions of di- and trichlorinated homologs that greatly exceeded those from the reference area. Although trichlorinated homologs were significantly higher in eggs from the contaminated area than in those from the reference area, the opposite was true in adult whole blood. These lower-chlorinated congeners are the most volatile and mobile congeners as well as the most easily metabolized and excreted by organisms. Thus, their spatial distribution and relative concentrations in different tissues may not reflect local contaminant conditions. This pattern also was true for heptachlorinated homologs. Because we cannot absolutely identify the source of PCBs delivered to eggs, we are unable to speculate about this apparent anomaly. The greater dominance of the tetra- through hexachlorinated homologs in eggs and blood from the contaminated area compared to the reference area, however, is consistent with the enrichment of the contaminated area sediments with Aroclor 1242. The elevated proportions of hepta- through decachlorinated congeners in the reference area eggs and whole blood most likely reflect general background contamination rather than a localized input of a particular mixture. Contributions of the heavier congeners in eggs and whole blood at the contaminated site most likely reflect background contamination, a small proportion of heavy congeners present in the Aroclor 1242 mixture, as well as other Aroclor mixtures used by the GE plants.

Despite the limited number of paired samples of blood from females and their eggs, it appears that maternal circulating PCB concentrations are positively correlated with those in eggs. It is important to note that blood collections were made at the time of nesting. Thus, egg formation had been completed, and the compounds detected in the blood may not necessarily reflect those circulating at the time of follicular growth. Over the course of the annual reproduction cycle of turtles, ovarian follicles undergo two periods of development [34]. The majority of follicular enlargement occurs from midsummer through late fall, with final follicular development and enlargement, shelling, and ovulation occurring in spring after emergence from hibernation. In the painted turtle (*Chrysemys picta*), energetic investment in initial development of ovarian follicles is derived from recently harvested food sources, whereas energy input for posthibernation derives from stored body lipids [35].

Because similar studies specifically examining the bioenergetics of egg production on snapping turtles have not been conducted, some question remains whether lipids and asso-

ciated lipophilic contaminants in eggs of this species originate from maternal reserves or from recently consumed dietary sources. Bishop et al. [7] observed that larger/older female snapping turtles or those with larger clutches from a contaminated site in Ontario did not produce eggs significantly higher in contaminant concentrations relative to those produced by smaller individuals. Those authors concluded that lipophilic egg contaminants were derived from the proximate diet before egg production, although maternal body burdens were not measured. Pagano et al. [10] evaluated adipose, liver, and egg tissues from six female snapping turtles exposed to PCBs and other lipophilic contaminants and concluded that the lower-chlorinated pattern in eggs compared to those of adipose tissue and liver suggests the proximate diet as the major contributor to egg stores. Those authors noted, however, that congener patterns in female tissues and eggs suggest that at least some portion of egg burdens were derived from maternal reserves.

Our observation of a weak positive trend of increasing egg PCB concentrations with female body size in the contaminated area does not necessarily provide compelling evidence that female somatic stores were the source of PCBs deposited in eggs. The positive relationships between blood PCB concentrations and size of females and males, however, suggests that circulating PCB concentrations reflect, at least in part, PCB stores established over long exposure periods (unless larger individuals in the contaminated area consistently consume prey items with a higher PCB concentration than smaller individuals do). Because *C. serpentina* clutch size generally increases with female size [36], our findings that PCB concentrations increased with increasing clutch size add further credence to a significant role of somatic sources for egg contaminants. Russell et al. [37], using a field-validated, fugacity-based model to assess partitioning of PCBs in snapping turtles and other oviparous species, determined that dietary lipids rapidly adopted the same lipid-based contaminant concentrations as somatic lipids such that eggs receive contaminant concentrations similar to maternal tissues rather than prey items.

Recent studies of maternal PCB transfer in snapping turtles [31] and of organochlorine pesticides in the American alligator (*Alligator mississippiensis*) [38] provide evidence that contaminants stored in somatic tissues rather than derived from the diet at the time of egg formation are the primary source of contaminants in eggs. These studies demonstrated significant relationships between total maternal body burdens and that of eggs as well as a nearly 1:1 egg to plasma ratio of lipophilic contaminants. Dabrowska et al. [31] found that while highly chlorinated congeners selectively partition into fat as K_{OW} decreases, the same is not true for partitioning between plasma and eggs, which is unaffected by K_{OW}. These findings agree with our data for 14 congeners and/or coeluting congeners found in sample pairs of maternal whole blood and eggs (Fig. 5), for which no trend of increasing egg to blood ratios with increasing chlorination was found. Thus, whereas somatic lipids may contain higher proportions of heavier-chlorinated congeners, light- to moderate-chlorinated congeners reach near–steady state equilibrium between fat reserves, blood components, and eggs regardless of lipid or contaminant content of recently harvested food items. Ultimately, the exact nature of the dynamics of contaminant body burden transfer to offspring in snapping turtles can be determined only in controlled laboratory experiments, such as those conducted in birds fed diets containing isotopically labeled PCBs [39].

The large number of animals captured over the three years of the present study provided the opportunity to explore potential relationships between adult morphology and PCB contamination. We measured metrics of sexual dimorphism to assess the possibility that PCBs may have altered sexual development in males, both because PCBs possess estrogenic activities [14] and because male reptiles inhabiting regions contaminated with organic compounds (including PCBs) have been found to display feminized traits [8,15,16]. The present results, however, showed no relationships between secondary sexual traits (PCL:CL and penis size:CL) and area of capture, nor were there relationships between blood PCB concentrations in males and these metrics.

Our data for gross morphology of adult turtles suggest that exposure to PCBs in the study areas did not alter the expression of secondary sexual traits or body size in the portion of the population sampled. We cannot, however, rule out the possibility that other effects of PCBs might occur in the contaminated area, particularly sublethal effects not examined in the present study. Certainly, we cannot assess the status of the population based on only three years of study and our nonrandom animal collection protocol, in which we targeted active nesting areas. Thus, we cannot speculate whether phenomena such as remobilization of PCBs during periods of fasting and subsequent toxicological effects [40], or other unknown effects, occur in the study area. Nor do the data presented here allow an assessment of the potential effects of maternal transfer of PCBs on health or performance of embryos and juveniles. Experimental studies using eggs collected during the present study to assess the effects of maternally derived PCBs on offspring health and fitness, however, were subsequently conducted, the results of which will be presented elsewhere.

SUPPORTING INFORMATION

Table S1. Polychlorinated biphenyl (PCB) congeners and respective homolog groups for PCB analyses conducted on snapping turtle (*Chelydra serpentina*) egg and blood tissues. Congeners are listed in order of elution off a 5% phenyl-methyl silicone DB-5MS capillary column (length, 60 m; inner diameter, 0.25 mm; film thickness, 0.25 μm); coeluting congeners are listed on the same line separated by a comma.

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