Population status of freshwater turtles across a PCB contamination gradient

James P. Gibbs[1],[*], Shahrokh Rouhani[2], Leyla Shams[2]

[1]**Department of Environmental and Forest Biology, Illick Hall,**
State University of New York College of Environmental Science and Forestry, Syracuse, NY 13210, USA
[2]**NewFields, Atlanta, GA 30309, USA**

ABSTRACT: Remarkably few studies on the effects of PCBs on wild turtles have been conducted. We contrasted population-level parameters of 2 species of freshwater turtles, the snapping turtle *Chelydra serpentina* and the painted turtle *Chrysemys picta*, across a strong PCB concentration gradient in the upper Hudson River, New York State, USA. Our study employed standardized capture methods that yielded 465 turtles during 1968 trap-nights over 2 yr (2006 and 2007) at 246 trapping sites. Individuals of both turtle species were relatively heavier in a contaminated river segment, but PCB concentration did not otherwise influence relative abundance of turtles, probabilities of habitat occupancy or detection, sex or age ratios, body size, incidence of deformities, external parasite occurrence, or several movement parameters in a manner consistent with adverse impacts to wild turtles. This lack of spatial trend in parameters of wild populations of aquatic turtles across a strong gradient of PCB contamination contrasts with predictions of adverse impacts derived from population modeling, dose-response studies, and tissue residue analyses.

KEY WORDS: PCB · Hudson River · Populations · Snapping turtle · Painted turtle

INTRODUCTION

The upper Hudson River in New York State, USA, was contaminated by the discharge of polychlorinated biphenyls (PCBs) from 2 electric capacitor plants in Fort Edward and Hudson Falls between the 1940s and 1977, after which further seepage and erosion of remnant deposits and contaminated banks continued to release PCBs (US EPA 2000). Fish and wildlife resources of the area are considered to have been adversely affected by the contamination. In the case of aquatic turtles, PCB concentrations in snapping turtle *Chelydra serpentina* tissues are elevated by 2 orders of magnitude in the contaminated section of the upper Hudson River (averaging ca. 3 ppm in eggs) relative to an uncontaminated segment immediately upriver (averaging ca. 0.02 ppm; Hudson River Natural Resource Trustees 2005). Given an

80% reduction in egg hatching success in snapping turtles predicted to occur at 3.3 ppm (Eisenreich et al. 2009), PCB levels observed in the contaminated segment of the river are considered to pose 'risk' to aquatic turtles (NOAA 2013).

Remarkably few studies on PCB exposure and effects on wild turtles have been conducted (Weir et al. 2010, Salice et al. 2014). Dose-response studies provide an avenue of inquiry and indicate that PCBs can cause adverse impacts to individual turtles (e.g. Eisenreich et al. 2009, Holliday et al. 2009, Holliday & Holliday 2012, Yu et al. 2012). Such studies can be advantageous for isolating the effect of exposure level to a particular contaminant on the performance of specific traits of individuals; however, implications of such studies of population-level endpoints remain unclear. Wild populations are the unit of biological organization most related to societal values (Selcer

[*]Corresponding author: jpgibbs@esf.edu

2006). Without an understanding of the demographic implications of reproduction- and survival-related anomalies in individuals reported in laboratory-based, dose-response studies, contaminant-mediated population-level effects cannot be assumed (Akça-kaya et al. 2008). Population modeling offers an alternative for exploring contaminant impacts under more field-relevant contexts. For example, Salice et al. (2014) concluded through deterministic population projection models that persistent contamination by PCBs in the upper Hudson River and the reduction it apparently causes in juvenile snapping turtle survival (Eisenreich et al. 2009) could translate into negative effects on the abundance of adult snapping turtles.

Field-based assessments of turtle population status across contamination gradients, although rarely undertaken, provide yet another and more direct approach for assessing population-level impacts as long as habitat covariates are controlled for. As is often the case with evaluations of contamination impacts on riverine biota, the geography of the PCB-impacted segment of the upper Hudson River is a complex array of aquatic and terrestrial habitats juxtaposed with urban-to-rural gradients of land use (Vispo & Knab-Vispo 2011). This mix of ecosystem heterogeneity and anthropogenic disturbances confronts turtles with complex mixtures of contaminants from agricultural and residential as well as industrial sources in the larger context of severe loss and fragmentation of habitats due to agriculture and urban development, with major potential impacts on turtle populations (Rizkalla & Swihart 2006).

In this study, we used a field-based assessment of turtle population status in relation to PCB exposure while controlling for habitat covariates to assess Salice et al.'s (2014) prediction that prolonged PCB exposure would reduce aquatic turtle population abundance in the upper Hudson River. To do so, we sampled aquatic turtle populations and habitat parameters up- and downriver of the source of PCB contamination in the Hudson River and within the sampling frame employed by the Hudson River Natural Resource Trustees in its assessments of contaminant loads in aquatic turtles and other river biota (NOAA 2013). These river segments varied strongly and consistently in terms of PCB concentrations in turtle eggs. Snapping turtle eggs in the uncontaminated, upriver 'reference' segment had an average PCB concentration 187 ± 182 (SD) ppb, whereas the PCB-contaminated segment spanned 3 regions with substantially elevated PCB concentrations: Region 1 at 9840 ± 10 400 (SD) ppb, Region 2 at 6330 ± 7710 ppb,

and Region 3 at 4290 ± 1690 ppb (regions designated by and estimates obtained from the Hudson River Natural Resource Trustees 2005).

Our study focused on 2 species of aquatic turtles, snapping turtles and painted turtles *Chrysemys picta*, because these were the best represented elements of the reptile community in the upper Hudson River and are frequent targets of toxicological studies (Bergeron et al. 1994, Bell et al. 2006), including in the Hudson River (Hudson River Natural Resource Trustees 2005, Kelly et al. 2008, Eisenreich et al. 2009, Salice et al. 2014). In addition to examining whether PCB exposure was associated with reduced turtle abundance, we assessed whether sex ratios were female-biased due to the feminizing effects of PCBs on developing embryos (Bergeron et al. 1994), whether individual fitness was reduced as reflected in lower body condition, and whether physical deformities were more prevalent (Bell et al. 2006). These studies were complemented by a study of snapping turtle movement ecology to infer the extent to which aquatic turtles in the river segments studied were demographically distinct and hence represented statistically independent population segments.

MATERIALS AND METHODS

This study's sampling frame was shoreline habitats along a ca. 100 km segment of the upper Hudson River between Waterford, New York, northwards and upriver to the confluence of Sacandaga and Hudson Rivers at Lake Luzerne. Within this area, exclusions were made for areas unsafe to navigate by small boat: a 3 km long swift water section between Hudson Falls and Fort Edward and short segments 100 m up- and downriver of all dams and locks. Within the sampling frame, 246 sampling sites ≥100 m apart were established at random locations along the river's shoreline. Inter-trap distances were chosen to achieve sample independence later validated via spatial autocorrelation analysis of turtle relative abundance implemented via the 'ncf' package in R (Bjornstad 2016, see 'Results'). Turtles were trapped at each sampling site during each of 2 years of study: between 28 May and 13 August 2006 and between 26 May and 4 August 2007 (pre-dating remediation efforts including dredging within the river initiated in 2009). Trapping dates coincided with the peak of annual activity in these species (Gibbs et al. 2007) and were scheduled in an alternating manner among river segments to avoid conflating any seasonal bias in turtle capture probability with river position.

Turtle populations were sampled using the most widely used method for monitoring and assessing freshwater turtle populations: baited hoop nets (Mali et al. 2014). Baited hoop nets have well-known biases (e.g. Ream & Ream 1966) but provide valid inference about differences in population status if deployed consistently among populations being contrasted. Each net comprised 3 hoops (3 ft [~90 cm] in diameter) covered with 1 inch (2.54 cm) mesh size netting and anchored to the river substrate with wooden poles. Traps were baited at deployment with the contents of a 100 g can of sardines packed in vegetable oil. All traps were checked daily and removed 5 d later, yielding 4 'trap nights' per sampling site per year. For all turtles captured, straight-line carapace length was measured with tree calipers (±1 mm) from the tip of the cervical scute posterior to the tip of the most distal marginal scute. Mass was measured with a Pesola® digital hanging scale. All individuals were inspected for visible ectoparasites (leeches) and external deformities following criteria outlined by Bell et al. (2006). All turtles captured were marked by notching the right supracaudal marginal scute; only initial captures were included in analyses.

Habitat conditions were characterized at each turtle trapping site. Water quality variables measured at each trap's entrance whenever it was checked included turbidity (measured by Secchi disk depth, to the nearest cm), temperature, and specific conductance (with an Oakton Waterproof pH 300 Meter). River bank slope nearest to the trap location was visually estimated. Substrate composition was estimated in July 2007 at all trapping sites as the fraction of points dominated by rocks or gravel versus muck, sand, clay, or vegetation sampled at 2 m intervals along a line parallel to the shoreline and intersecting with the trap and extending from 10 m upriver to 10 m downriver of each trapping site for 10 points in total. Availability of potential basking platforms was estimated from counts of logs, rocks, or other objects unattached to the shore, protruding above the water surface, able to support a basking turtle, and occurring within 10 m of a trap. River morphology associated with each trapping site was characterized in terms of river width, mean river depth, and river depth variability (the coefficient of variation of river depth measurements). To do so, a perpendicular transect was established that extended between the trapping site and the opposite shoreline. River depth was measured along this transect at 10 m intervals. Position was determined with a Bushnell Yardage Pro Sport 450 laser rangefinder aimed at a reflecting object (a 1 × 1 m piece of sheet metal) placed at the transect origin on the river bank. River depth at each sampling point was measured with a HawkEye Handheld Digital Depth Sounder. Last, length of shoreline and extent of land cover types (aggregate vegetated wetland, agriculture, developed land, and forest as a fraction of the non-open water area) within a 100 m radius circular area centered on each trapping site were estimated from the New York State Area Hydrography dataset (1:24 000) and the National Land Cover Dataset 2001 (Homer et al. 2007, resolution 30 m), respectively, both obtained from the New York State GIS Clearinghouse.

A complementary study of snapping turtle movement ecology was conducted to examine whether the samples of aquatic turtles in the 2 population segments contrasted were indeed independent (if intermixing of turtles was high, our samples would not reflect distinct populations with consistently different PCB exposure histories) and because movement is an important behavioral attribute potentially affected by PCB exposure (cf. Deem et al. 2009). Radio-transmitters were attached to 22 individuals captured in baited hoop nets in June and July 2008: 11 turtles (3 females and 8 males) in the reference river segment and 11 turtles (2 females and 9 males) in the contaminated river segment. Sex ratios of animals marked reflected the typical male-bias in hoop net samples of aquatic turtles (Ream & Ream 1966) and were matched to the extent possible upstream versus downstream within the total sample of 22 turtles – the largest sample we could track with a field crew of 2 full-time technicians available. Turtles were fitted with 30 g transmitters (model AI-2F, Holohil Systems) with a battery life of 18 mo following the recommendations of Boarman et al. (1998) and re-located with a Yagi directional antenna in conjunction with an R-1000 telemetry receiver (Communications Specialists); each turtle's geographical coordinates (±2 m) were then determined using a Garmin eTrex handheld 12-channel global positioning system device. Individuals were located once every 1 to 2 wk from the time of capture until mid-September in 2008 and then again between late May and mid-September 2009 (that is, across 2 peak activity seasons and 2 nesting seasons), after which we recaptured all turtles, removed their radios, and then released the animals. Contrasts of turtle population parameters were predicated on a null expectation of independence of any given population parameter's estimate relative to river segment (contaminated versus reference).

Generalized linear mixed modeling (GLMM) (McCulloch et al. 2008) was conducted using IBM SPSS Statistics 23 (www.ibm.com/analytics/us/en/

technology/spss/) to contrast relative abundance between river segments while controlling for environmental variables and year. The dependent variable in GLMM was the reported turtle counts at each trap in a given year, treated as a site-specific repeated measurement. Independent variables of turtle sex/age and trap ID were treated as site-specific attributes, whereas other variables such as temperature, river width, river depth, and PCB concentrations were considered as site-specific covariates. GLMM assumes a specific distribution for the dependent variable (in our study Poisson and negative binomial distributions of turtle capture success) and uses maximum likelihood estimation procedures to predict the model parameters and their associated significance.

Turtle survey data were also analyzed using site-occupancy models (MacKenzie et al. 2002) to differentiate between the probabilities of site occupancy (ψ), colonization (γ) and extinction (ϵ), and species detection (p) by incorporating site and sampling covariates using Program PRESENCE version 11.5 (MacKenzie 2012). Site and sampling covariates included region (upriver reference, region 1, 2, or 3), median PCB concentration, and average PCB concentration (derived from Hudson River Natural Resource Trustees 2005). The Simple Multi-Season Model (MacKenzie 2012) was used for the analysis. Model outputs included season- and site-specific occupancy and colonization probabilities, as well as site- and sampling-specific detection probabilities, with seasons referenced to 2006 (4 trap nights per trap site) and 2007 (also 4 trap nights per trap site). Prior to the analysis, categorical variables were replaced by their equivalent indicator variables, i.e. 1 when the category matched and 0 otherwise. We assessed a large subset of possible models that directly addressed the study's a priori hypothesis, that is, site occupancy (ψ) and detection probability (p) were affected by PCB exposure as indexed by region (upriver reference, and Regions 1, 2, or 3), or median PCB concentration and average PCB concentration. Akaike's information criterion (AIC) of each model was computed and ranked; models with ΔAIC (the difference between a particular model AIC and the minimum AIC) smaller than 2 were considered equivalent (Burnham & Anderson 2002).

Population parameters based on frequencies—traps capturing turtles, females in sample, adults, individuals above median turtle length in the entire river system (an index of population size structure), deformities, and hosting leeches—were contrasted between river segments using the G statistic for the

log-likelihood ratio goodness of fit test with Williams' correction for continuity (Sokal & Rohlf 1995). Thresholds used for size at maturity (classification as 'adult') were ≥20 cm carapace length for snapping turtles and ≥11 cm carapace length for painted turtles (Ernst et al. 1994).

Body condition (relative mass per unit length) for a given turtle was assessed using a nonparametric generalized additive mixed modeling approach (Hastie & Tibshirani 1990), which enabled controlling for the nonlinear relationship between body mass and body size while partitioning out the effects of the categorical covariates sex/age and river position (using the R package gamm4, Wood 2009).

Evaluation of the influence of snapping turtle sex, body size (carapace length), and river segment on snapping turtle movement parameters was undertaken through an ANCOVA (Field 2009) using IBM SPSS Statistics 23 in which turtle sex and river segment were treated as fixed factors, with body size and turtle movement parameters as the covariate and dependent variable, respectively.

Mean values of turtle habitat parameters were contrasted between the contaminated and reference river segments with a Student's 2-tailed t-test for independent samples.

All tests with probabilities $\alpha \leq 0.05$ were interpreted as 'significant.'

RESULTS

Riverine habitats associated with turtle trapping sites in the contaminated river segment differed significantly ($p < 0.05$) from those in the reference segment mostly in terms of characteristics of uplands surrounding the river: a 400% increase in extent of agriculture nearby, a 167% increase in amount of development nearby, and a 32% decrease in extent of forest (Table 1), along with a 113% increase in water conductivity, a 40% decrease in substrate rockiness, a 38% decrease in turbidity, a 29% decrease in bank slope, a 23% decrease in river depth, a 10% decrease in river width, a 9% increase in river depth variation, and a 4% increase in water temperature (Table 1).

Total sampling effort for turtles equaled 1968 trap-nights over 2 yr at the 246 trapping sites. Pooled across sites and years, trapping 'success' averaged 11.3 snapping turtles and 4.2 painted turtles per 100 trap nights. In 2006, we captured a total of 225 turtles (Table 2): mostly snapping turtles (82% of total captures) and painted turtles (17%) as well as 3 map tur-

Table 1. Habitat attributes at 246 turtle trapping sites within the upper Hudson River contrasted with Student's t-tests between a PCB-uncontaminated reference segment (upriver of Glens Falls, New York) and a contaminated segment (downriver of Fort Edward, New York). Data are mean ± SD

Variable	Reference (n = 103)	Contaminated (n = 143)	t	p
Turbidity (m)	2.75 ± 0.67	1.70 ± 0.66	12.3	0.001
Temperature (C°)	23.40 ± 1.88	24.39 ± 2.38	3.4	0.001
Conductivity (mmho cm^{-1})	79.33 ± 24.87	169.41 ± 50.19	15.9	0.001
Bank slope (%)	0.59 ± 0.30	0.42 ± 0.20	5	0.001
Substrate (% rock or gravel)	0.55 ± 0.61	0.33 ± 0.56	2.8	0.004
Basking platforms (within 10 m)	1.96 ± 3.01	2.66 ± 3.43	1.6	0.097
River depth (m)	3.72 ± 2.10	2.88 ± 1.22	3.6	0.001
River width (m)	206.45 ± 85.65	185.33 ± 68.28	2.1	0.032
River depth variation (CV)	0.53 ± 0.13	0.58 ± 0.12	2.5	0.012
Wetland (within 100 m)[a]	0.05 ± 0.13	0.03 ± 0.10	1.0	0.324
Development (within 100 m)[a]	0.03 ± 0.08	0.08 ± 0.15	3.2	0.002
Agriculture (within 100 m)[a]	0.02 ± 0.06	0.1 ± 0.13	6.3	0.001
Forest (within 100 m)[a]	0.38 ± 0.20	0.26 ± 0.16	5.1	0.001
Length shoreline within 100 m (m)	239.36 ± 96.12	233.08 ± 85.53	0.5	0.589

[a]Extent as fraction of total area

tles *Graptemys geographica*, 2 stinkpots *Sternotherus odoratus*, and a single wood turtle *Glyptemys insculpta*. In 2007, the same 246 sites were resampled, yielding a total of 240 turtles, mostly snapping turtles (62%) and painted turtles (32%), as well as 3 map turtles and 10 stinkpots (Table 2). Only 13 snapping turtles and 2 painted turtles were recaptured during the study; all analyses reported are based on initial captures only.

Relative abundance of aquatic turtles was uncorrelated at the 100 m inter-trap distance implemented, suggesting that trapping sites represented independent samples: mean Moran's *I* for total captures over both years was 0.020 (−0.243 to 0.262, 95% CI) for snapping turtles and 0.020 (−0.218 to 0.227, 95% CI) for painted turtles.

When related to time (year) and environmental variables at capture sites via a GLMM, traps disproportionately captured male snapping turtles and did so in segments of the river that were wider, shallower, and surrounded by more open water (Table 3A). Relative abundance of painted turtles also was higher where the river was wider and where more extensive wetland, basking platforms, and turbid waters occurred (Table 3A). Notably, river position (upriver or reference versus downriver or contaminated) had no influence on capture success of either snapping or painted turtles. When the same analysis was conducted including mean PCB concentration in turtle eggs as determined for the 4 river segments designated by the Hudson River Natural Resource Trustees (2005), outcomes were similar; most notably,

mean PCB concentration estimated in a particular river segment did not affect relative abundance of either turtle species (Table 3B).

Occupancy models (Table 4), all with high estimated goodness of fit, indicated that, for snapping turtles, river association (upriver or downriver), mean PCB concentration, or median PCB concentration did not consistently affect probabilities of habitat occupancy or detection, with model-averaged estimates of probabilities of habitat occupancy (0.4–0.6) and detection (0.15–0.20) among top (ΔAIC <2 units) models comparable among river segments (Table 5). Similarly, for painted turtles the single top-ranked model (Table 4), which had a very high estimated goodness of fit, did not include region, mean PCB concentration, or median PCB concentration as a determinant of probability of habitat occupancy (0.20–0.25, Table 5), although it did include region as a co-variate of detection probability (ranging from 0.00–0.40), with painted turtles showing among the lowest and high-

Table 2. Number of individuals of aquatic turtle species captured in traps in the upper Hudson River during 2006 and 2007. Values reflect initial turtle captures; all re-captures were excluded from analyses

Species	River segment	Year		
		2006	2007	Both
Snapping turtle	Contaminated	116	107	223
	Reference	64	43	107
	Entire	180	150	330
Painted turtle	Contaminated	25	58	83
	Reference	14	19	33
	Entire	39	77	116
Wood turtle	Contaminated	0	0	0
	Reference	1	0	1
	Entire	1	0	1
Map turtle	Contaminated	3	3	6
	Reference	0	0	0
	Entire	3	3	6
Stinkpot	Contaminated	2	10	12
	Reference	0	0	0
	Entire	2	10	12
Total		225	240	465

est detection probabilities in the river segments with elevated PCB contaminations (Table 5).

All other turtle population parameters estimated were independent of river position in both 2006 and in 2007 and for both snapping turtles and painted turtles (Table 6) with 3 exceptions: a higher proportion of female snapping turtles was captured in the reference segment in 2007, a higher proportion of large male snapping turtles was captured in the contaminated segment in 2006 and in 2006/2007 combined, and a higher proportion of female painted turtles was captured in the reference river segment in both 2007 and in 2006/2007 combined. Notably, no patterns in physical deformities, of a moderate or minor degree, in individual turtles were evident in contrasts of aquatic turtle populations in contaminated versus reference river segments (Table 6), nor were major deformities observed in either river segment.

Generalized additive models explained much of the variation in turtle mass (91% in snapping turtles, 84% in painted turtles); after controlling for carapace length, male painted turtles were lighter than females and individuals of both species were heavier in the contaminated than in the reference river segment (Table 7).

Mean cumulative distance traveled by 22 snapping turtles across successive relocations over the 1.5 yr tracking period was 3.00 ± 1.92 km (SD; min. = 0.92, max. = 9.68) for a daily rate of mean = 0.04 ± 0.02 km d^{-1} (min. = 0.01 max. = 0.12). Snapping turtles remained close to initial capture sites over 2 seasons: the average maximum distance among any relocation of any given turtle during the 2 seasons was just 0.93 ± 0.62 km (min = 0.31, max = 3.37). The distance moved between the first and last capture as a fraction of cumulative distance moved across all relocations was just 0.18 ± 0.14 km (min. = 0.03 max. = 0.67). Notably, snapping turtles, which were all initially captured in the river's main channel, subsequently remained mostly within the main channel itself: an average of 0.77 ± 0.34 (min. = 0.1 max. = 1.0) of total relocations of each individual were within the river channel. When turtles left the river, the average maximum distance from the river's shore was 0.12 ± 0.19 km (min. = 0.10 max. = 0.70). ANCOVA indicated independence across the sample of 22 turtles tracked in terms of cumulative distance moved and daily movement rate in relation to turtle sex, size (carapace length), and location in the contaminated versus reference river segment (Table 8). Although our intent was not to measure snapping turtle survival rates, it is notable that no marked turtle died over 2 seasons of monitoring in either river segment.

Table 3. Generalized linear mixed models explaining relative abundance (capture success) of snapping turtles and painted turtles at 246 trapping sites along a 100 km extent of the upper Hudson River (see 'Methods' for variable descriptions). Analyses were structured using 2 approaches: (A) upriver (uncontaminated) / downriver (contaminated) contrast, and (B) average PCB concentration contrast among 4 river segments (regions) as designated by the Hudson River Natural Resource Trustees (2005) with strongly varying average PCB in turtle eggs: Reference = average PCB concentration of 187 ppb (n = 103 trapping sites), Region 1 = 9843 ppb (n = 41 trapping sites), Region 2 = 4986 ppb (n = 64 trapping sites), and Region 3 = 4287 ppb (n = 38 trapping sites). The upriver (uncontaminated) segment included the Reference segment as designated by the Hudson River Natural Resource Trustees (2005) whereas the downriver (contaminated) segment included Regions 1, 2, and 3. **Bold** values indicate significant coefficients (p ≤ 0.05). Data are mean ± SD

(A) Average PCB concentration not included		
Model	Snapping turtle Poisson	Painted turtle Negative binomial
Independent variables		
Gender/age = female	**−2.852 ± 0.507**	0.056 ± 0.323
= juvenile	**−1.517 ± 0.476**	−
= male	0[a]	0[a]
Position = contaminated	−0.694 ± 2.215	−7.744 ± 4.427
= reference	0[a]	0[a]
River width	**0.004 ± 0.002**	**0.007 ± 0.003**
River depth (mean)	**−0.148 ± 0.074**	−0.327 ± 0.184
River depth (variation)	0.746 ± 0.785	1.931 ± 1.22
Water	**0.019 ± 0.005**	0.010 ± 0.009
Wetland	0.011 ± 0.006	**0.039 ± 0.009**
Development	−0.03 ± 0.026	−0.005 ± 0.033
Agriculture	−0.045 ± 0.044	−0.236 ± 0.197
Shoreline	0.001 ± 0.001	−0.002 ± 0.001
Bank slope	−0.000 ± 0.005	−0.019 ± 0.010
Basking platforms	0.036 ± 0.019	**0.13 ± 0.032**
Rock/gravel substrate	−0.004 ± 0.002	−0.035 ± 0.016
Temperature	−0.003 ± 0.079	−0.025 ± 0.154
Conductivity	−0.007 ± 0.004	−0.011 ± 0.010
Turbidity	0.283 ± 0.204	**1.389 ± 0.359**
(B) Average PCB concentration included		
Model	Snapping turtle Poisson	Painted turtle Poisson
Independent variables		
Gender/Age = female	**−1.649 ± 0.188**	−0.090 ± 0.227
= juvenile	**−1.975 ± 0.216**	−
= male	0[a]	0[a]
Position = contaminated	−0.153 ± 0.383	−0.652 ± 1.120
= reference	0[a]	0[a]
PCB	0.000 ± 0.000	−0.000 ± 0.000
River width	0.000 ± 0.001	−0.000 ± 0.001
River depth (mean)	0.022 ± 0.044	−0.024 ± 0.099
River depth (variation)	0.536 ± 0.503	0.201 ± 0.831
Water	**0.018 ± 0.005**	**0.022 ± 0.009**
Wetland	**0.015 ± 0.006**	**0.045 ± 0.009**
Development	0.004 ± 0.007	−0.001 ± 0.016
Agriculture	−0.004 ± 0.007	0.018 ± 0.011
Shoreline	0.000 ± 0.001	−0.003 ± 0.001
Bank slope	−0.002 ± 0.003	−0.017 ± 0.010
Basking platforms	0.034 ± 0.019	**0.110 ± 0.040**
Rock/gravel substrate	−0.003 ± 0.002	−0.015 ± 0.010
Temperature	0.044 ± 0.032	**0.371 ± 0.081**
Conductivity	**0.004 ± 0.001**	**0.011 ± 0.003**
Turbidity	−0.035 ± 0.115	−0.459 ± 0.250

[a]Redundant coefficient is set to 0

Table 4. Occupancy models for estimating site occupancy (ψ), detection (p), colonization (γ), and local extinction (ε) for 2 fresh-water turtle species (snapping turtle and painted turtle) associated with 246 trapping sites in the upper Hudson River each sampled over 4 d in both 2006 and 2007. Weight is a measure of support for each model; k denotes number of parameters and −2LL denotes −2 log likelihood. Site and sampling covariates include: Region (upstream reference, and Regions 1, 2, or 3) and average and median PCB concentration in turtle eggs in each river segment (derived from Hudson River Natural Resource Trustees 2005)

No.	Model	AIC	ΔAIC	AIC weight	Model	k	−2LL likelihood	Chi-squared	p	Model R^2
Snapping turtle										
1	ψ(.),γ(.),p(.)	1407.4	0	0.215	1	5	1397.4	2.008	1.000	0.999
2	ψ(Region),γ(.),p(.)	1407.7	0.26	0.189	0.878	8	1391.7	3.166	1.000	0.961
3	ψ(Region),γ(Region),p(.)	1408.8	1.35	0.109	0.509	11	1386.8	3.213	1.000	0.963
4	ψ(.),ϒ(.),p(AveragePCB)	1409.1	1.69	0.092	0.430	6	1397.1	2.076	1.000	0.998
5	ψ(AveragePCB),γ(.),p(.)	1409.1	1.7	0.092	0.427	6	1397.1	2.110	1.000	0.995
6	ψ(.),γ(.),p(MedianPCB)	1409.3	1.9	0.083	0.387	6	1397.3	2.037	1.000	0.998
7	ψ(MedianPCB),γ(.),p(.)	1409.4	2	0.079	0.368	6	1397.4	2.009	1.000	0.999
8	ψ(.),γ(.),p(Region)	1410.6	3.23	0.043	0.199	8	1394.6	2.308	1.000	0.993
9	ψ(AveragePCB),γ(.),p(AveragePCB)	1411.1	3.68	0.034	0.159	7	1397.1	2.080	1.000	0.997
10	ψ(MedianPCB),ϒγ(.),p(Region)	1412.8	5.36	0.015	0.069	9	1394.8	2.314	1.000	0.993
11	ψ(AveragePCB),γ(.),p(Region)	1412.8	5.37	0.015	0.068	9	1394.8	2.506	1.000	0.984
12	ψ(Region),γ(.),p(Region)	1412.8	5.38	0.015	0.068	11	1390.8	3.102	1.000	0.963
13	ψ(MedianPCB),γ(.),p(MedianPCB)	1412.9	5.54	0.014	0.063	7	1398.9	2.128	1.000	0.997
14	ψ(Region),γ(Region),p(Region)	1414.2	6.82	0.007	0.033	14	1386.2	3.308	1.000	0.960
Painted turtle										
1	ψ(.),γ(.),ε(.),p(Region)	518.74	0	0.961	1.000	7	504.7	3.756	1.000	1.000
2	ψ(Region),γ(.),p(.)	526.72	7.921	0.018	0.0191	6	514.7	4.702	1.000	0.954
3	ψ(Region),γ(.),ε(.),p(.)	526.73	7.935	0.018	0.0189	6	514.7	4.831	1.000	0.954
4	ψ(AveragePCB),γ(.),p(.)	532.53	13.686	0.001	0.0011	5	522.5	6.356	1.000	0.970
5	ψ(MedianPCB),γ(.),ε(.),p(.)	534.54	15.688	0.000	0.0004	5	524.5	32.444	1.000	0.903
6	ψ(.),γ(MedianPCB),p(.)	535.02	16.221	0.000	0.0003	6	523.0	2.689	1.000	0.997
7	ψ(.),γ(AveragePCB),p(.)	535.09	16.293	0.000	0.0003	6	523.1	2.602	1.000	0.998
8	ψ(.),γ(Region),ε(Region),p(.)	535.19	16.589	0.000	0.0002	9	517.2	2.721	1.000	0.989
9	ψ(.),γ(Region),p(.)	536.50	17.760	0.000	0.0001	7	522.5	2.446	1.000	0.997
10	ψ(.),γ(MedianPCB),ε(MedianPCB),p(.)	536.91	18.175	0.000	0.0001	7	522.9	2.813	1.000	0.996
11	ψ(.),γ(AveragePCB),ε(AveragePCB),p(.)	537.26	18.518	0.000	0.0001	7	523.3	2.659	1.000	0.997
12	ψ(AveragePCB),γ(.),ε(.),p(.)	537.69	18.840	0.000	0.0001	5	527.7	42.970	1.000	0.916
13	ψ(.),γ(.),ε(.),p(.)	537.98	19.135	0.000	0.0001	5	528.0	2.163	1.000	0.945
14	ψ(.),γ(.),p(.)	537.98	19.135	0.000	0.0001	5	528.0	3.756	1.000	1.000
15	ψ(.),γ(.),ε(.),p(AveragePCB)	538.82	20.025	0.000	0.0001	6	526.8	1.440	1.000	0.994
16	ψ(.),γ(.),p(AveragePCB)	538.82	20.025	0.000	0.0001	6	526.8	1.440	1.000	0.994
17	ψ(.),γ(.),ε(.),p(MedianPCB)	539.11	20.317	0.000	0.0001	6	527.1	6.196	1.000	0.995
18	ψ(Region),γ(Region),ε(Region),p(.)	543.14	24.470	0.000	0.0001	8	527.1	19.875	1.000	0.918

DISCUSSION

This study took advantage of the upper Hudson River as a site where PCB concentrations were among the highest reported in the US (Baker et al. 2006) to examine whether PCB exposure could cause adverse, population-level impacts to wild turtles in a manner both substantive and additive to other anthropogenic impacts that typically confront reptiles in the environment (Gardner et al. 2007). Our population assessment of aquatic turtles was robust insofar as it involved a large number of independent study sites (n = 246) sampled over 2 yr (for a total of almost 2000 trap-nights) via a long-established, standardized, and widely-employed capture technique (baited hoop nets). We assumed that detection prob-ability (cf. MacKenzie et al. 2002) of both species of turtles did not vary between river segments, which occupancy modeling bore out for snapping turtles and apparently for painted turtles given the lack of any clear relationship between detection probabilities and PCB exposure (lowest in the most contaminated river segment, highest in the second-most contaminated segment, and intermediate in the uncontaminated and third-most contaminated segments). Movement analyses of snapping turtles confirmed that the 2 primary population samples contrasted between contaminated and reference river segments were likely independent given the observed sedentariness of snapping turtles. In sum, the study did not reveal consistent variation in population-level parameters in wild populations of either

Table 5. Parameter estimates (±SE) from occupancy models for estimating site occupancy (ψ), detection (p), colonization (γ), local extinction (ε) for 2 freshwater turtle species (snapping turtle and painted turtle) associated with 246 trapping sites in the upper Hudson River each sampled over 4 d in both 2006 and 2007

Location	Parameter	Snapping turtle	Painted turtle
Reference	ψ_{2006}	0.452 ± 0.055	0.233 ± 0.074
	ψ_{2007}	0.543 ± 0.085	0.207
	p_{2006}	0.200 ± 0.024	0.095 ± 0.038
	p_{2007}	0.146 ± 0.021	0.167 ± 0.052
	γ	0.260 ± 0.104	0.085 ± 0.063
	ε	0.064	0.392 ± 0.183
Region 1	ψ_{2006}	0.438 ± 0.069	0.233 ± 0.074
	ψ_{2007}	0.528 ± 0.093	0.207
	p_{2006}	0.204 ± 0.030	0 ± 0
	p_{2007}	0.148 ± 0.026	0 ± 0
	γ	0.313 ± 0.115	0.085 ± 0.063
	ε	0.142	0.392 ± 0.183
Region 2	ψ_{2006}	0.519 ± 0.068	0.233 ± 0.074
	ψ_{2007}	0.606 ± 0.083	0.207
	p_{2006}	0.202 ± 0.026	0.253 ± 0.093
	p_{2007}	0.147 ± 0.023	0.395 ± 0.078
	γ	0.250 ± 0.124	0.085 ± 0.063
	ε	0.025	0.392 ± 0.183
Region 3	ψ_{2006}	0.468 ± 0.063	0.233 ± 0.074
	ψ_{2007}	0.559 ± 0.093	0.207
	p_{2006}	0.202 ± 0.026	0.101 ± 0.054
	p_{2007}	0.147 ± 0.023	0.177 ± 0.078
	γ	0.252 ± 0.114	0.085 ± 0.063
	ε	0.046	0.392 ± 0.183

snapping turtles or painted turtles associated with PCB exposure, which presumably has been prolonged given the average age of reproducing female snapping turtles of 34 to 40 yr in northern populations (Galbraith et al. 1988, Congdon et al. 1994) and that reduction of PCB levels in the river did not occur substantially until after our study was concluded, i.e. in 2009 with the initiation of site remediation (dredging) activities.

Radio-telemetry studies suggested that snapping turtles moved very little, ranging on average <1 km over 2 activity seasons from their initial capture point. This degree of sedentariness is consistent with other reports that ranging behavior in snapping turtles is minimal (Kiviat 1980, Obbard & Brooks 1981, Galbraith et al. 1987). Despite the occurrence of hydrologically connected and unconnected wetland along the river's length, snapping turtles primarily remained in the river itself (75% of all re-locations). Snapping turtle population status in the upper Hudson River is therefore likely heavily influenced by habitat conditions within the river itself, including PCB exposure levels, as opposed to those of off-river

wetlands (Bowne et al. 2006). Although we did not include painted turtles in our movement comparison, painted turtles appear to be even less mobile than snapping turtles in this region (Patrick & Gibbs 2010), although other studies suggest that painted turtles occupying rivers elsewhere in North America can make extended movements (>20 km) over a single active season (MacCulloch & Secoy 1983).

Although river segment or PCB exposure did not influence capture success for either species examined, we did identify habitat variables affecting variation in turtle abundance. More snapping turtles were trapped at sites with more open-water habitats and shallower and wider segments of the river. Painted turtles showed a similar response except that they were more abundant at sites with increased availability of palustrine wetlands nearby, more basking platforms, and higher water turbidity. Both snapping and painted turtles are known to use a variety of permanent or semi-permanent lentic and lotic waters (e.g. ponds, lakes, marshes, swamps, ditches, and occasionally salt marshes) within their ranges, including significantly polluted and eutrophic habitats (snapping turtles: Galbraith et al. 1988, Brown et al. 1994; painted turtles: DeCatanzaro & Chow-Fraser 2010). For painted turtles, the association with greater numbers of basking platforms likely reflects their need to bask regularly, which is critical for increasing body temperature above ambient water temperatures in order to increase metabolic rates and rate of energy assimilation to ensure fat accumulation for reproduction and overwintering, which is particularly important at the northern edge of these species' ranges, which include the upper Hudson River (Gibbs et al. 2007).

Our study primarily implemented an upriver/ downriver experimental design as do many assessments of contaminant effects on wildlife, including PCB impacts on the biota of the upper Hudson River (NOAA 2013). While this study design can be considered an example of 'pseudo-replication' (sensu Hurlbert 1984), landscape-scale ecological studies such as this one do not generally permit classical experimentation because the treatment cannot be replicated (Hargrove & Pickering 1992). Nevertheless, within our study area we explicitly considered environmental covariates that also might drive variation in our response variables between the 2 treatment areas, which enabled development of a correlational understanding between turtle population response and environmental characteristics including PCB exposure.

Habitat differences that we observed between upriver and downriver sites (Table 1) emphasize the

Table 6. Contrasts of population parameters for snapping turtles and painted turtles in a PCB-contaminated versus reference segment of the upper Hudson River in 2006 and 2007 and in both years combined

Parameter	Year	%(n) Contaminated	Reference	G(adj)	df	p
Snapping turtle						
Traps capturing	2006	61.5 (143)	70.9 (103)	2.3	1	0.128
no turtles	2007	59.4 (143)	69.9 (103)	2.8	1	0.092
Females[a]	2006	21.8 (101)	19 (58)	0.2	1	0.675
	2007	8.1 (99)	21.1 (38)	3.9	1	0.048
	Combined	15 (200)	19.8 (96)	1.0	1	0.308
Adults[a]	2006	87.1 (116)	90.6 (64)	0.5	1	0.476
	2007	92.5 (107)	88.4 (43)	0.6	1	0.437
	Combined	89.7 (223)	89.7 (107)	0.0	1	0.993
Males >	2006	62 (79)	42.6 (47)	4.5	1	0.035
median length[b]	2007	48.4 (91)	33.3 (30)	2.1	1	0.151
	Combined	54.7 (170)	39 (77)	5.3	1	0.022
Females >	2006	45.5 (22)	45.5 (11)	0.0	1	1.000
median length[b]	2007	50 (8)	50 (8)	0.0	1	1.000
	Combined	46.7 (30)	47.4 (19)	0.0	1	0.962
Deformed[c]	2006	75.9 (116)	76.6 (64)	0.0	1	0.916
	2007	72 (107)	69.8 (43)	0.1	1	0.790
	Combined	74 (223)	73.8 (107)	0.0	1	0.975
Hosting leeches	2006	2.6 (116)	3.1 (64)	0.0	1	0.843
	2007	9.3 (107)	7 (43)	0.2	1	0.643
	Combined	5.8 (223)	4.7 (107)	0.2	1	0.666
Painted turtle						
Traps capturing	2006	90.2 (143)	92.2 (103)	0.3	1	0.585
no turtles	2007	89.5 (143)	87.4 (103)	0.3	1	0.608
Females[a]	2006	10.3 (107)	23.3 (43)	2.7	1	0.102
	2007	44.8 (58)	47.4 (19)	0.0	1	0.849
	Combined	44.6 (83)	57.6 (33)	1.6	1	0.210
Males >	2006	71.4 (14)	75 (4)	0.0	1	0.896
median length[b]	2007	37.5 (32)	30 (10)	0.2	1	0.672
	Combined	47.8 (46)	42.9 (14)	0.1	1	0.748
Females >	2006	54.5 (11)	80 (10)	1.4	1	0.229
median length[b]	2007	23.1 (26)	77.8 (9)	8.0	1	0.005
	Combined	32.4 (37)	78.9 (19)	11.0	1	0.001
Deformed[c]	2006	68 (25)	64.3 (14)	0.1	1	0.818
	2007	62.1 (58)	78.9 (19)	1.9	1	0.172
	Combined	63.9 (83)	72.7 (33)	0.8	1	0.361
Hosting leeches	2006	4 (25)	7.1 (14)	0.1	1	0.711
	2007	15.5 (58)	31.6 (19)	2.1	1	0.150
	Combined	12 (83)	21.2 (33)	1.4	1	0.230

[a]Based on estimates of size at maturity (see 'Materials and methods')
[b]Median length (straight-line carapace length) for entire sample of male snapping turtles = 313 mm, for female snapping turtles = 285 mm, for male painted turtles = 136 mm, and for female painted turtles = 159 mm
[c]Turtles only with minor or moderate deformities (cf. Bell et al. 2006), as no turtles with major (lethal) deformities were captured

need to control for environmental conditions inherent to river segments when using upriver/downriver experimental designs, which most research on PCB impacts on Hudson River biota do not (NOAA 2013). More specifically, the downriver segment had more

agriculture and urban development on upland landscapes nearby trapping sites, elevated water conductivity (higher ionic concentration), less rocky substrates at trapping sites, clearer waters, reduced extent of forest on adjacent river banks, lower bank slopes, shallower river depths, narrower river width, more consistent river depth, and slightly warmer water temperatures (Table 1). Some of these differences may reflect increased nutrients and human-generated organic waste in water bodies in more developed landscapes, which may benefit freshwater turtles in terms of enhanced food sources and aquatic vegetation (Knight & Gibbons 1968, Souza & Abe 2000). Without simultaneously controlling for environmental conditions when contrasting populations with different histories of PCB exposure levels at the spatial scale of river segments, as we did with GLMMs of relative abundance (Table 3), ecological influences on turtle abundance could have been conflated with contaminant impacts.

Environmental factors also affected traits of individual turtles. After controlling for carapace length, individuals of both turtle species were heavier in the contaminated than the reference river segment, and a higher proportion of large male snapping turtles was found in the contaminated than in the reference segment in 2006 and in 2006/2007 combined (Table 6). Greater relative mass is generally considered to reflect greater individual fitness in reptiles (Wikelski & Romero 2003) insofar as relatively heavier turtles have more reserves to allocate for growth, reproduction, and overwintering. The lower reaches of the river with their slower, warmer waters and greater nutrient levels (as indexed by higher overall water conductivity, Table 1) may represent more productive habitats in terms of food availability and opportunity for mass gain given the affinity (and presumably higher fitness) of both snapping and painted turtles for warmer waters and more eutrophic habitats (Galbraith et al. 1988, De-Catanzaro & Chow-Fraser 2010). Notably, any spatial pattern in physical deformities, of moderate, minor, or major degree was lacking, an outcome consistent

Table 7. Summary of generalized additive models fitted to snapping turtles (n = 330) and painted turtles (n = 116) captured in the upper Hudson River in 2006 and 2007, to explore relationships between body mass and age/sex and river segment (PCB-contaminated versus reference) while controlling for individual turtle length

Parameter	Parametric coefficients				Nonlinear effects (nonparametric)		
	Estimate	SE	*t*	p	df	*F*	p
Snapping turtle (deviance explained = 91.4%)							
Constant	8.036	0.205	39.074	<0.001			
Sex/age (female vs. juvenile)	−0.605	0.470	−1.287	0.199			
Sex/age (female vs. male)	−0.350	0.213	−1.638	0.102			
River segment (contaminated vs. reference)	−0.693	0.154	−4.482	<0.001			
Carapace length					6.487	340.6	<0.001
Painted turtle (deviance explained = 84.5%)							
Constant	0.410	0.010	42.253	<0.001			
Sex (female vs. male)	−0.034	0.015	−2.220	0.0284			
River segment (contaminated vs. reference)	−0.043	0.011	−4.068	<0.001			
Carapace length					2.814	68.56	<0.001

Table 8. ANCOVA of snapping turtle movement parameters in relation to river segment (reference versus contaminated) and individual turtle sex and carapace length for 22 turtles tracked over 2 activity seasons in the upper Hudson River

Dependent variable	Source	Type III SS	df	MS	*F*	p
Average daily movement	Corrected model	0.001	3	0.000	0.383	0.767
	Intercept	0.002	1	0.002	3.803	0.067
	River segment (contaminated versus reference)	0.000	1	0.000	0.591	0.452
	Sex	0.000	1	0.000	0.107	0.747
	Carapace length (cm)	0.000	1	0.000	0.690	0.417
	Error	0.011	18	0.001		
	Total	0.046	22			
	Corrected total	0.012	21			
Cumulative distance moved	Corrected model	4.465	3	1.488	0.367	0.778
	Intercept	4.113	1	4.113	1.014	0.327
	River segment (contaminated versus reference)	0.116	1	0.116	0.029	0.867
	Sex	4.400	1	4.400	1.085	0.311
	Carapace length (cm)	0.038	1	0.038	0.009	0.924
	Error	72.992	18	4.055		
	Total	275.345	22			
	Corrected total	77.457	21			

with other reports of a lack of manifestation of external deformities in field-sampled populations of adult freshwater turtles exposed to PCBs and other contaminants elsewhere (Bell et al. 2006, Davy & Murphy 2009).

Operational sex ratio is a parameter of particular interest as an indicator of potential PCB impacts because PCB exposure has been suggested as a feminizing agent in turtles (Bergeron et al. 1994). A higher proportion of female snapping turtles was observed in the reference river segment in 2007, and a higher proportion of female painted turtles was observed in the reference river segment in 2007 and in 2006/2007 combined, patterns in contrast to expectations of PCB-exposure as a feminizing agent in

turtle populations. An alternate explanation relates to nest site choice and temperature-dependent sex determination because higher incubation temperatures generally yield increasing fractions of females in both painted (Schwanz & Janzen 2008) and snapping turtles (Rhen & Lang 1994). However, reduced forest cover and more extensive agricultural lands in the contaminated river segment (Table 1) would be expected to generate warmer incubation conditions for turtle nests (Schwanz & Janzen 2008) and a higher, not lower, female fraction. The most likely explanation for the lower female fraction in the contaminated river segment is higher vulnerability to road mortality in female turtles. The contaminated river segment was far more urbanized, with an asso-

ciated denser road network and heavier traffic (Table 1). Female turtles are killed disproportionately on roads because they undertake annual nesting migrations that males do not, resulting in male-biased sex ratios in more urbanized areas (Steen & Gibbs 2004, Steen et al. 2006), a pattern we observed in this study.

In conclusion, this assessment of population parameters of aquatic turtles across a gradient of PCB contamination in the upper Hudson River, an environmental issue of nation-wide significance (Baker et al. 2006), failed to identify any contaminant-induced alteration of multiple, population-level endpoints in wild turtles. Turtles are late-maturing, long-lived, and largely sedentary higher-order predators, attributes that may make them useful meters of bio-accumulation of contaminants (Bishop et al. 1998) but poor indicators of contaminant effects on populations. This is because changes in the demographic parameters perhaps most sensitive to contaminant-associated effects, such as clutch size, egg survival, and hatchling survival (e.g. Salice et al. 2014), are also those to which population growth in turtles is least sensitive (Congdon et al. 1993, 1994). The lack of spatial trends we observed in population parameters of aquatic turtles associated with a strong gradient of PCB contamination contrasts with predictions of likely occurrence of adverse demographic impacts derived from population projection modeling (Salice et al. 2014), dose-response studies (Holliday et al. 2009), and tissue residue analyses (NOAA 2013). More field assessments *in situ* with well designed studies of clearly defined population-level endpoints will be needed to understand levels of PCB exposure that generate substantive adverse effects on wild populations of aquatic turtles.

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