Environ. Sci. Technol. 2009, 43, 6052–6057

# Latent Mortality of Juvenile Snapping Turtles from the Upper Hudson River, New York, Exposed Maternally and Via the Diet to Polychlorinated Biphenyls (PCBs)

KAREN M. EISENREICH, SHANNON M. KELLY, AND CHRISTOPHER L. ROWE*

*University of Maryland Center for Environmental Science, Chesapeake Biological Laboratory, PO Box 38, Solomons, Maryland 20688*

*Received March 19, 2009. Revised manuscript received May 31, 2009. Accepted June 5, 2009.*



We conducted a factorial experiment to compare sublethal and lethal responses of juvenile snapping turtles exposed maternally and/or through the diet to polychlorinated biphenyls (PCBs) over 14 months posthatching. Maternal exposure did not affect embryonic development or hatching success. Thyrosomatic indices were not influenced by treatments, although hepatosomatic indices were lower in animals having been exposed to PCBs maternally relative to those having been exposed both maternally and via the diet. Dietary PCB exposure reduced metabolic rates of juveniles in two of three assays conducted. Approximately eight months after hatching, high rates of mortality began to emerge in individuals having been exposed maternally to PCBs, and mortality rate correlated with $[PCB]_{total}$ in eggs. Prior to death, individuals that died experienced lower growth rates than those that survived, suggesting chronic effects prior to death. By 14 months posthatching, only 40% of juveniles derived from females in the contaminated area had survived, compared to 90% from the reference area. Such latent effects of maternally derived contaminants suggest that assessments of environmental impacts based upon shorter-term studies may provide very conservative estimates of the severity of effects, as they cannot capture responses that may emerge later in the life cycle.

## Introduction

Snapping turtles (*Chelydra serpentina*) have widely served as biomonitors as they have the propensity to bioaccumulate contaminants and transfer lipophilic contaminants to their eggs (*1*–*6*). Thus, eggs provide useful indicators for establishing the presence of contaminants in local systems that contain species that are prone to maternal transfer. As well as serving as biomonitors, snapping turtles have been employed as models to examine effects on development that may result from maternal transfer of contaminants in species of long life span and high trophic position (*7*). Effects included decreased hatching success and increased incidence of deformities in hatchlings (*8*–*11*). While numerous contaminants have been shown to induce effects on offspring following maternal transfer, those most often implicated have been organochlorine pesticides, polychlorinated biphenyls (PCBs), and in some cases polybrominated diphenyl ethers (PBDEs *4*, *7*–*9*, *11*).

As many aquatic habitats harbor complex mixtures of contaminants, it is often difficult to identify the particular compound(s) that may pose the greatest risk to resident species. For example, research suggests a correlation between exposure to PCBs and developmental anomalies in turtles, yet often the habitats studied have been contaminated by other compounds (*4*, *8*, *9*, *11*). However, there exist some systems in which contamination by PCBs is so severe that exposure to PCBs likely outweighs exposure to other, background contaminants. The upper Hudson River harbors some of the highest PCB concentrations in the U.S. (*12*). Contamination of the upper Hudson River is the result of historical discharge of PCBs from two electric capacitor plants operated by the General Electric Corporation, as well as from erosion of remnant deposits and seepage from bedrock fractures below the plants (*13*). Due to the extensive contamination of the upper Hudson River by PCBs, this system provides a rare opportunity to examine responses of wildlife to PCB exposure.

PCBs act as endocrine disrupting compounds (EDCs) that have several modes of action. PCBs and their metabolites can be estrogenic, antiestrogenic, or may exhibit both properties (*14*–*16*). PCBs can also alter the thyroid system, interfering with the transport, metabolism and receptor interactions of thyroid hormones (*17*–*19*). Alteration of thyroid hormone-modulated responses may result in abnormal metabolic function during the critical embryonic and juvenile stages of development. As a result, thyroid-disrupting compounds such as PCBs may alter energy assimilation efficiency, energy allocation to somatic and reproductive tissue growth, and immune response, potentially affecting survival and reproductive fitness (*20*–*23*).

While the effects of maternal transfer of PCBs on reptiles have been characterized, these assessments typically focused only on embryonic survival and development or traits of newly hatched individuals (*1*, *3*, *4*, *6*, *24*). There remains little information regarding the potential for effects to emerge long after direct exposure of embryos via maternal transfer has ceased, or as a result of exposure of juveniles via consumption of contaminated food. Thus, the possible latent effects of embryonic exposure that may emerge during the juvenile period, alone and in combination with ongoing dietary exposure of juveniles, are unknown. If effects emerge beyond the scope in duration of most studies, estimates of environmental impacts based on those studies will likely be conservative. Subsequently, regulatory and management activities based upon results from relatively short-term studies may not be sufficient to protect ecosystem health or to develop successful restoration/remediation strategies.

We assessed the long-term effects of maternally transferred PCBs as well as proximate effects of exposure to food-borne PCBs in juvenile snapping turtles collected from the upper Hudson River, NY. From early egg development through the ensuing 14 months of the juvenile period, we quantified development, metabolism/growth, and survival in individuals exposed or unexposed to maternally derived PCBs, food-borne PCBs, and their combination.

## Experimental Section

**Egg Collection and Incubation.** Eggs were collected from areas designated as either "reference" or "contaminated" in northern New York. The contaminated area was a section of

* Corresponding author phone: (410) 326-7227; fax: (410) 326-7302; e-mail: rowe@cbl.umces.edu.



10.1021/es9008344 CCC: $40.75 
Published on Web 07/02/2009

the Hudson River just downstream of the Hudson Falls and Fort Edward General Electric facilities (43° 18′ N, 73° 35′ W and 43° 16′ N, 73° 35′ W, respectively), including several areas known to be "hot spots" of sediment contamination (average PCB concentration >40 mg/kg wet weight). The reference area consisted of lakes and ponds that were north and west of the contaminated area, distant from significant and direct sources of PCBs. Eggs from both areas were collected from nests or through induction of egg laying via intraperitoneal injection of females with oxytocin (*25*); clutches from oxytocin induced females were represented in both areas. Eggs were measured for diameter and wet weight. Three eggs per clutch were frozen at −20 °C for analysis of PCB concentrations. Detailed information on selection of study areas and collection methods is provided in Kelly et al. (*6*).

Eggs were incubated at 25 °C, a temperature known to produce only males (*26*). Moisture and humidity were maintained by misting the eggs and nest substrate (vermiculite) with water at 2−3 day intervals. Eggs were periodically candled to determine viability and development. Hatchlings were measured for carapace length ("CL") and wet weight ("ww"), examined for gross abnormalities, and assigned to groups for subsequent 14-month studies of juveniles.

**Posthatching Protocol.** For 14 months posthatching ("mph"), we assessed potential effects of maternal contribution of PCBs, food-borne exposure to PCBs, and the combination. We used a total of 144 hatchlings derived from 16 total egg clutches collected from females from the contaminated ($n = 6$) and reference areas ($n = 10$). Individuals from all clutches were used in assessing all end points with the exception of mortality, for which only those clutches having produced five or more hatchlings were used (contaminated $n = 5$; reference $n = 6$). Smaller clutches were excluded from mortality comparisons as they would have hindered analysis of these proportional data. Small clutch sizes resulted from limited numbers of viable eggs from natural nests due to predation as well as from induction of females via oxytocin, which often produces partial clutches. Mean (±1 SE) total PCB concentrations were 3953 ± 1454 and 61 ± 8 ng/g ww (varying from 75 to 9220 and 21 to 86 ng/g ww) in the contaminated and reference area eggs, respectively. The eggs used for assessing mortality had concentrations of 3546 ± 1710 and 55 ± 10 ng/g ww in the contaminated and reference areas (varying from 75 to 9220 and 21 to 78 ng/g ww respectively). Hatchlings from each area were divided into two additional treatments, to be fed a diet of natural prey items collected from either area (see below). Thus there were four treatments for the remainder of the study, designated hereafter as the maternal (collection) area followed by food type: contaminated area, contaminated food = "CC"; contaminated area, reference food = "CR"; reference area, contaminated food = "RC"; reference area, reference food = "RR".

Hatchlings were held in a 20 °C laboratory prior to a simulated overwintering period. To mimic the winter-time period of dormancy that occurs in temperate climates, we overwintered hatchlings beginning in mid October by incrementally lowering temperature to a final temperature of 5 °C, and incrementally increasing temperatures beginning in late February. Turtles were maintained at 22−25 °C for the remainder of the study. Each individual received a passive integrated transponder (PIT) tag following methods described by Rowe and Kelly (*27*), providing for identification of individuals, which were held in groups (below) per a common-garden design. Dietary exposures began following overwintering when yolk stores were depleted and hatchlings began to display interest in food. Thus dietary exposures were approximately eight months in duration.

Food consisted of aquacultural gelatin (Aquatic Ecosystems, Inc., Apopka, FL) mixed with ground fish and invertebrates collected in the study areas and frozen for subsequent use. Food collected from the contaminated area and reference area contained mean total PCB concentrations of 5955 ± 427 ng/g and 83 ± 6 ng/g ww respectively. Food was provided ad libitum and was analyzed by bomb calorimetry to verify caloric content (reference: 4291 cal/g; contaminated: 3525 cal/g).

For each food treatment, we used eight replicate tanks (57 L) containing 5 L of water and nine to ten turtles (four to five from each area). As tanks were designated only by food treatment (not by collection area), each received animals from both areas (e.g., tanks in which animals were fed contaminated food contained only individuals of treatments CC and RC, and tanks designated to receive reference food contained only CR and RR).

**Growth Rate, Size, and Metabolic Rates.** Instantaneous growth rate was calculated on ww and CL as [(ln(ww or CL final)-ln(ww or CL after overwinter))/199 days]. Sizes were measured monthly. Metabolic rates were measured bimonthly, beginning in May (9 mph), on a subset of 16 individuals from each treatment (representing all tanks; the same individuals were used during all assays). Resting metabolic rate (25 °C) was measured as oxygen consumption by resting, fasted animals at 2 h intervals over a period of 12 h by microrespirometry (Micro-Oxymax; Columbus Instruments, Columbus, OH) following a 24 h acclimation period (*28*).

**Sex Ratios, Histopathology, and Organosomatic Indices.** At the conclusion of the experiment, ww and CL measurements were made and individuals were euthanized by inhalation of isoflorane followed by decapitation. Animals were dissected for inspection of the gonads for determination of sex (*26*) and abnormalities in gonadal organization. Those not defined as male were morphologically female (e.g., not intersex). We calculated hepatosomatic and thyrosomatic indices (HSI and TSI) as [(liver or thyroid gland weight/body weight) × 100]. Individuals that died during the experiment were excluded since they may have remained in the tanks for up to approximately 12 h after death, possibly influencing organ and carcass wet weights.

**Analysis of PCB Concentrations.** Detailed PCB extraction and quantification methods were presented by Kelly et al. (*6*). Briefly, three eggs from each clutch were rinsed and the contents were composited and homogenized. Eggs were randomly selected from nests as research has shown little within-clutch variation in contaminant concentrations (*1*). A 5 gram sample of the homogenate from each clutch was dried with sodium sulfate, spiked with surrogate standards, and Soxhlet extracted for 24 h using dichloromethane. Five gram samples of each food type were similarly processed. Extracts were subsampled to gravimetrically measure lipid content. Gel permeation chromatography was used to remove lipids followed by Florisil cleanup. Laboratory blanks were processed with and in the same manner as all tissue samples by extracting 50 g of sodium sulfate spiked with surrogate standards.

Total PCB concentrations represent the sum of 110 individual congeners and/or groups of coeluting congeners (*6*). Samples were quantified using a Hewlett-Packard 5890 gas chromatograph equipped with a $^{63}$Ni electron capture detector, with hydrogen as the carrier gas and nitrogen as the makeup gas. A 60 m × 0.25 mm 5% phenyl-methyl silicon DB-5MS capillary column was used with inlet pressure of 100 kPa. The oven temperature program was 100 °C hold for two min, ramp to 170 at 4 °C/min, 170−280 at 3 °C/min, and a final 5 min hold at 280 °C. The injector temperature was held at 225 °C and the detector at 285 °C. Information on

**TABLE 1. Wet Weight, Diameter, And Lipid Content of Eggs and Wet Weight and Carapace Length (CL) of Hatchlings and Post-Overwintered Juveniles from Contaminated and Reference Areas. Values Are Means ±1 SE**

| | egg size | | | size at hatching | | size postoverwintering (7 mph) | |
|---|---|---|---|---|---|---|---|
| | wet weight (g) | diameter (mm) | lipid (%) | wet weight (g) | CL (mm) | wet weight (g) | CL (mm) |
| contaminated | 8.67 ± 0.10 | 26.5 ± 0.1 | 4.93 ± 0.82 | 7.24 ± 0.36 | 27.0 ± 0.6 | 7.50 ± 0.12 | 26.7 ± 0.2 |
| reference | 9.59 ± 0.19 | 25.8 ± 0.2 | 7.04 ± 0.23 | 8.37 ± 0.42 | 29.3 ± 0.5 | 8.41 ± 0.18 | 28.4 ± 0.3 |
| | $P < 0.001$ | $P = 0.006$ | $P = 0.009$ | $P < 0.001$ | $P = 0.006$ | $P < 0.001$ | $P < 0.001$ |

surrogates, internal standards, and recoveries can be found in Kelly et al. (*6*).

**Statistical Analyses.** Mortality, growth rate, size by month, and PCB concentrations were analyzed on tank means by analysis of variance (ANOVA) followed by Tukey's pairwise comparisons. When assessing size by month, initial size was defined as size following overwintering. Mortality data were transformed by arcsine square root. Bimonthly metabolic rates were analyzed on tank means by analysis of covariance (ANCOVA) using ww as the covariate. Size and metabolic data collected over the duration of the study were also analyzed using repeated measures ANOVA. Relationships between PCB concentrations in eggs and juvenile mortality were assessed by linear regression. Statistical significance was judged based upon a type I error rate of $\alpha = 0.05$. Prior to statistical analyses, data were tested to verify that assumptions of the models were met.

## Results

**Eggs and Hatchlings.** Eggs from the reference area were heavier and had higher lipid content than those from contaminated area, but were smaller in diameter (Table 1). Hatchlings from reference eggs were larger than those from the contaminated area, both in terms of ww and CL (approximately 13 and 8% larger, respectively; Table 1). For contaminated and reference areas respectively, neither time to hatching (84.7 ± 0.330 and 84.5 ± 0.240 days; $P = 0.625$) nor hatching success (73.2 and 66.8%; $P = 0.472$) differed.

**Juveniles.** There was a large divergence in survival among treatments beginning 8 − 9 mph (2 months following the overwintering period; Figure 1). Survival was affected only by area of egg collection (e.g., maternal transfer only; $P < 0.001$); feeding regime had no main effect nor was there an interaction between feeding regime and area of collection.

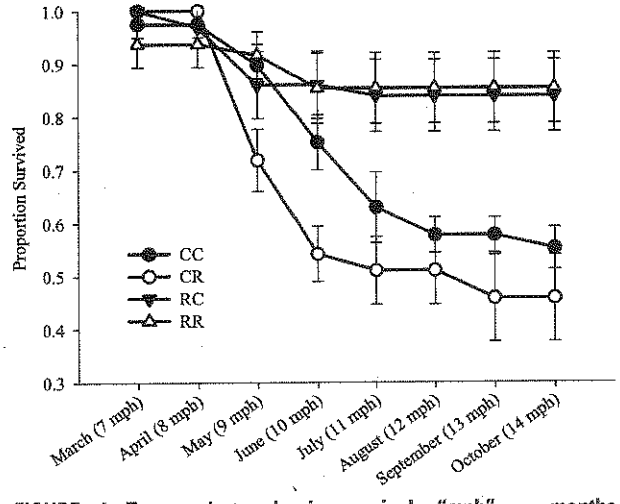


**FIGURE 1. Temporal trends in survival. "mph" = months posthatch. Symbols represent means ±1 SE. CC = Contaminated area and contaminated food; CR = Contaminated area and reference food; RC = Reference area and contaminated food; RR = Reference area and reference food.**

For juveniles from eggs from the contaminated area, those that died had received significantly higher maternal exposure than those that survived (3605 ± 481 versus 1404 ± 260 ng/g ww egg PCB, respectively; $P < 0.001$), but there was no such relationship for those from the reference area (58.1 ± 7.4 versus 63.1 ± 2.7 ng/g ww egg PCB, respectively; $P = 0.455$). There was a positive relationship between PCB concentration in eggs and juvenile mortality in the contaminated area ($R^2 = 0.864$; Figure 2). However, PCB concentration explained only 18% of the variation in mortality in the reference area (proportion died = 0.254 − 0.00191 clutch × PCB, $R^2 = 0.18$, $P = 0.0402$; not illustrated). There were no differences in instantaneous growth rate, measured as CL, among treatments ($P = 0.636$). Nor were there statistical differences in monthly CL measurements among treatments when analyzed over the duration of the study by repeated measures ($P = 0.059$). However, CL comparisons within months revealed that individuals from CC, CR, and RC were significantly smaller than those from RR in May (9 mph; $P = 0.001$, 0.026, and 0.026 respectively), whereas in June (10 mph) and July (11 mph) only individuals from CC and RC were smaller from those in RR (June $P < 0.001$ and $P = 0.023$; July $P = 0.007$ and 0.032 respectively; Figure 3). Within a treatment, individuals that died were smaller than those that survived although small sample sizes limited statistical power which may have precluded assigning statistical significance to the results. For those individuals that survived to the end of the study, there were no differences in size among any treatments ($P = 0.195$).

TSI did not significantly differ among treatments at $P < 0.05$, although TSI for individuals in CC appeared lower than TSIs in other treatments ($P = 0.086$; Table 2). HSI was significantly lower in CR than CC ($P = 0.045$; Table 2). Metabolic rates analyzed across the study by repeated measures ANOVA differed among treatments ($P < 0.001$). Metabolic rates in May (9 mph) and July (11 mph) were reduced in treatments CC and RC relative to CR and RR ($P < 0.001$). By the final measurement (13 mph) differences were

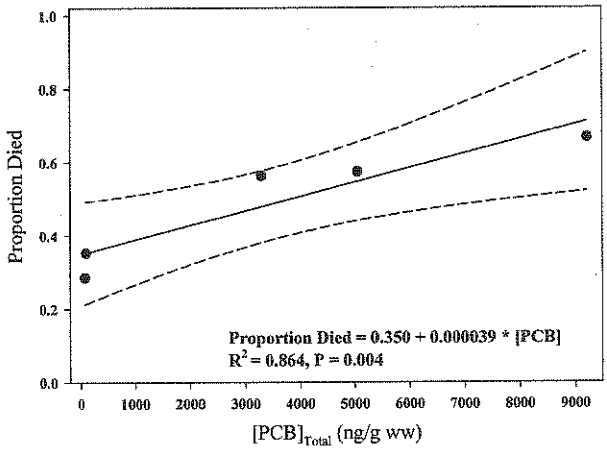


**FIGURE 2. Relationship between PCB concentrations (ng/g ww) in eggs collected in the contaminated area and mortality of juveniles having hatched from them. Dotted lines indicate 95% confidence interval.**

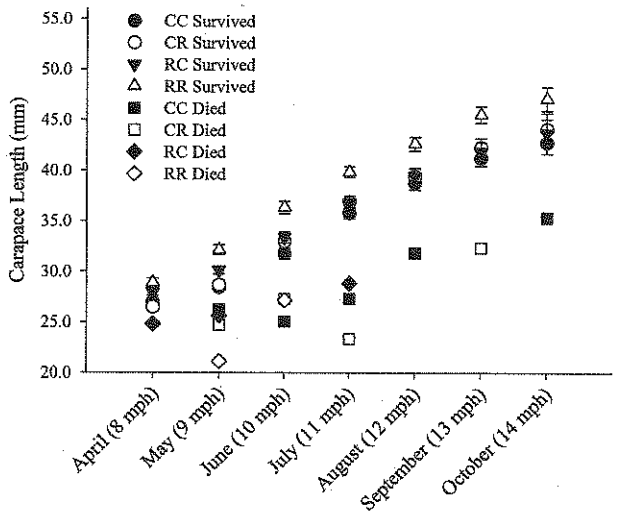


**FIGURE 3. Mean monthly carapace length of juveniles that survived or died. "mph" = months posthatch. Error bars represent 1 SE. CC = Contaminated area and contaminated food; CR = Contaminated area and reference food; RC = Reference area and contaminated food; RR = Reference area and reference food.**

no longer detected (Table 2). Dissections revealed that feminization (presence of ovarian rather than testicular tissue) was exceptionally rare. Ninety-nine and 100% of juveniles from the contaminated and reference area were morphologically male.

## Discussion

The most striking and unexpected result of this study was the latent onset of mortality of juveniles having been exposed to PCBs via maternal transfer. Beginning 8−9 months after hatching, juveniles from clutches in the contaminated area (CC and CR) began experiencing high rates of mortality in contrast to those from the reference area. Ultimately, only approximately 40% of individuals derived from the contaminated area survived the entire 14 month study versus 90% from the reference area. Animals that died were smaller compared to survivors in the same treatment at the time of death (Figure 3). Reduced sizes in these individuals may reflect reduced feeding as a result of physiological stress; we observed no evidence of size-specific competitive hierarchies for food (e.g., heightened aggression of larger animals directed toward smaller animals), nor was food unavailable at any time. Animals that died had only slightly increased in size from their initial size following overwintering, and in several cases the size at death was less than the initial size. Thus, the lack of a difference among treatments in size of turtles surviving to the end of the study most likely reflected the high mortality rate of small individuals from the contaminated area, such that only the largest survived the duration of the experiment.

Whereas studies with other species have reported correlations between embryonic mortality and egg PCB concentration (*29*), we observed little mortality of hatchlings prior to, during, and immediately following the overwintering period. Rather, mortality rates increased only after the juveniles reached eight to nine months of age. Mortality rates reflected maternal PCB contribution to eggs such that the highest rates were observed in individuals from the most contaminated clutches. There was only a weak relationship between mortality and PCB concentration for reference animals, reflecting the small numbers of individuals that died and the narrow range in PCB concentrations (21−78 ng/g ww) in eggs from that area. While we cannot rule out the possibility that other factors may have played a role in inducing mortality, due to the strong relationship between mortality and egg PCB concentration in the contaminated area, it seems unlikely that other compounds may have played a substantial role in mortality.

Eggs collected from the contaminated area produced significantly smaller hatchlings than those from the reference area. During the juvenile growth period, animals from the contaminated area (CC and CR) remained significantly smaller than reference area animals fed reference food (RR). Even after taking into account the differences in sizes following overwintering (Table 1), animals from the reference area fed contaminated food (RC) remained consistently smaller relative to those fed reference food (RR) until late in the study when mortality led to variability in average sizes and numbers of survivors among treatments, limiting statistical power in making comparisons.

We observed reductions in metabolic rates in treatments in which animals were fed contaminated food (CC and RC) relative to reference food only in the first two assays (9 and 11 mph), prior to the onset of high mortality rates. The third assay, in which we observed no differences in MR among treatments, was conducted after the highest mortality rates occurred in treatments CC and RC (13 mph). Thus only the healthiest animals survived to the third assay, suggesting that these individuals may not have been metabolically compromised relative to those that had previously died.

Reductions in metabolic rates in treatments CC and RC were consistent with reductions in growth rates of juveniles in these treatments during the same period, suggesting a correlation between metabolic function and growth. Similarities in metabolic rate among treatments at the end of the study correspond with the similarities in final sizes at this time. This result further suggests a relationship between MR and growth in the least healthy individuals, such that loss of these individuals from the population reduced their influence on mean responses of survivors measured at the end of the study.

**TABLE 2. Standard Metabolic Rate Measured on Three Dates and Hepatosomatic (HSI) and Thyrosomatic (TSI) Indices of Juveniles Measured at the End of the Study[a]**

| | standard metabolic rate ($\mu$L $O_2$/g × min) | | | | |
|---|---|---|---|---|---|
| treatment | May (9 mph) | July (11 mph) | September (13 mph) | TSI (%) | HSI (%) |
| CC | $0.92 \pm 0.058^{A}$ | $1.25 \pm 0.057^{A}$ | $1.49 \pm 0.020$ | $0.010 \pm 0.0009$ | $5.128 \pm 0.161^{A}$ |
| CR | $1.00 \pm 0.055^{B}$ | $1.32 \pm 0.067^{B}$ | $1.46 \pm 0.030$ | $0.012 \pm 0.0010$ | $4.577 \pm 0.140^{B}$ |
| RC | $0.81 \pm 0.057^{A}$ | $1.20 \pm 0.054^{A}$ | $1.51 \pm 0.020$ | $0.013 \pm 0.0008$ | $4.840 \pm 0.138^{AB}$ |
| RR | $0.97 \pm 0.059^{B}$ | $1.37 \pm 0.052^{B}$ | $1.50 \pm 0.020$ | $0.014 \pm 0.0011$ | $4.902 \pm 0.090^{AB}$ |
| | $P < 0.001$ | $P < 0.001$ | $P = 0.559$ | $P = 0.086$ | $P = 0.045$ |

[a] Different letters within columns indicate treatments that differed following post-hoc pairwise comparisons. Values are mean ±1 SE. "mph" = months post-hatch. CC = Contaminated area and contaminated food. CR = Contaminated area and reference food. RC = Reference area and contaminated food. RR = Reference area and reference food.

Hepatosomatic and thyrosomatic indices were inconsistent among treatments. Differences in TSI were expected as PCBs and their metabolites have been shown to cause thyroid hormone alterations that can lead to hypothyroidism and reduced glandular weights (*30, 31*). In addition, in other species exposure to PCBs causes a loss of body weight and mortality due to the degeneration of the liver (e.g., reduced HSI (*32*)). Thus we expected to observe differences in HSI particularly in light of the limited weight gain or weight loss in some treatments. As with our measures of growth and MR, our inability to detect differences among treatments in organosomatic indices may reflect the time at which the measurements were made. Calculating these indices required dissection of the animals, therefore they represent measurements made at the end of the experiment, after mortality of juveniles (having been excluded from the calculations) exposed to the highest concentrations of PCBs had occurred.

Given the high concentrations of PCBs to which embryos were exposed, we expected that monitoring multiple end points over a relatively long period of time would reveal sublethal effects that would not be observed in shorter term studies (such as those that span only the embryonic period). Aside from growth rate, metabolic rate was the only sublethal end point measured that we found to respond to PCB exposure, and it appeared to only be altered by proximate, dietary exposure rather than maternal exposure. Thus, under the conditions of this study, metabolic rate appears not to be an adequate indicator of maternal effects, thus would not be a sufficient metric for assessing organismal health in systems in which the primary route of exposure is via maternal transfer. Despite the numerous end points that we quantified, we are unable to suggest a putative mode of toxicity responsible for the latent onset of mortality of individuals exposed maternally to high concentrations of PCBs. Elucidating the underlying causes of the observed effects would require biochemical assessment of changes at the cell, tissue and organ levels that were beyond the scope of the study.

Maternally derived PCBs alone have not previously been shown to induce mortality in juvenile snapping turtles, although in combination with co-occurring contaminants embryonic mortality has been observed (*9, 11*). The design of our study provided the opportunity to identify effects of PCBs that may emerge long after hatching, beyond the scope of most studies of maternal transfer, allowing us to quantify latent effects that otherwise would remain uncharacterized. Moreover, our results suggest that some indicators of sublethal effects may either be wholly insensitive to maternally derived contaminants or reflect only effects of proximate (dietary) exposure, limiting their use as early warning signals of latent expression of maternal effects. Despite the logistical and financial challenges inherent to studies such as ours, the information they provide can be critical to developing cogent regulatory strategies in the light of dramatic responses to contaminants that may emerge long after maternal exposure has ceased.



## Acknowledgments

This study was supported by a research grant (009/03A) from the Hudson River Foundation, a graduate fellowship to S. Kelly from the Hudson River Foundation, and by the Gene Lane Endowment. During preparation of this manuscript, K. Eisenreich was supported by graduate fellowships granted by SETAC/Procter and Gamble and the USEPA STAR Graduate Fellowship Program (FP-91690301). This study benefited from the participation of K. Bogel, K. Hauselberger, T. Meaders, and K. Richardson. The information presented here has not been subjected to review by the supporting agencies, and no official endorsement should be inferred. Contribution 4297-CBL from the University of Maryland Center for Environmental Science.

## Literature Cited

(1) Bishop, C. A.; Brown, G. P.; Brooks, R. J.; Lean, D. R. S.; Carey, J. H. Organochlorine contaminant concentrations in eggs and their relationship to body size, and clutch characteristics of the female common snapping turtle (*Chelydra serpentina serpentina*). *Arch. Environ. Contam. Toxicol.* **1994**, *27*, 82–87.

(2) Bishop, C. A.; Ng, P.; Norstrom, R. J.; Brooks, R. J.; Pettit, K. E. Temporal and geographic variation of organochlorine residues in eggs of the common snapping turtle (Chelydra serpentina serpentina) (1981−1991) and comparisons to trends in the herring gull (*Larus argentatus*) in the Great Lakes Basin in Ontario, Canada. *Arch. Environ. Contam. Toxicol.* **1996**, *31*, 512–524.

(3) Pagano, J. J.; Rosenbaum, P. A.; Roberts, R. N.; Sumner, G. M.; Williamson, L. V. Assessment of maternal contaminant burden by analysis of snapping turtle eggs. *J. Great Lakes Res.* **1999**, *25*, 950–961.

(4) de Solla, S. R.; Fernie, K. J. Characterization of contaminants in snapping turtles (*Chelydra serpentina*) from Canadian Lake Erie Areas of Concern: St. Clair River, Detroit River, and Wheatley Harbour. *Environ. Pollut.* **2004**, *132*, 101–112.

(5) de Solla, S. R.; Fernie, K. J.; Letcher, R. J.; Chu, S. G.; Drouillard, K. G.; Shahmiri, S. Snapping turtles (*Chelydra serpentina*) as bioindicators in Canadian Areas of Concern in the Great Lakes Basin. 1. Polybrominated diphenyl ethers, polychlorinated biphenyls, and organochlorine pesticides in eggs. *Environ. Sci. Technol.* **2007**, *41*, 7252–7259.

(6) Kelly, S. M.; Eisenreich, K. M.; Baker, J. E.; Rowe, C. L. Accumulation and maternal transfer of polychlorinated biphenyls in snapping turtles of the upper Hudson River, New York, USA. *Environ. Toxicol. Chem.* **2008**, *27*, 2565–2574.

(7) Rowe, C. L. "The calamity of so long life": life histories, contaminants, and potential emerging threats to long-lived vertebrates. *BioScience* **2008**, *58*, 623–631.

(8) Bishop, C. A.; Brooks, R. J.; Carey, J. H.; Ng, P.; Norstrom, R. J.; Lean, D. R. S. The case for a cause-effect linkage between environmental contamination and development in eggs of the common snapping turtle (*Chelydra s. serpentina*) from Ontario, Canada. *J. Toxicol. Environ. Health.* **1991**, *33*, 521–547.

(9) Bishop, C. A.; Ng, P.; Pettit, K. E.; Kennedy, S. W.; Stegeman, J. J.; Norstrom, R. J.; Brooks, R. J. Environmental contamination and developmental abnormalities in eggs and hatchlings of the common snapping turtle (*Chelydra serpentina serpentina*) from the Great Lakes-St Lawrence River basin (1989−1991). *Environ. Pollut.* **1998**, *101*, 143–156.

(10) Bell, B.; Spotila, J. R.; Congdon, J. D. High incidence of deformity in aquatic turtles in the John Heinz National Wildlife Refuge. *Environ. Pollut.* **2006**, *142*, 457–465.

(11) de Solla, S. R.; Fernie, K. J.; Ashpole, S. Snapping turtles (*Chelydra serpentina*) as bioindicators in Canadian Areas of Concern in the Great Lakes Basin. II. Changes in hatching success and hatchling deformities in relation to persistent organic pollutants. *Environ. Pollut.* **2008**, *153*, 529–536.

(12) Baker, J. E.; Bohlen, W. F.; Bopp, R. F.; Brownawell, B.; Collier, T. K.; Farley, K. J.; Geyer, W. R.; Nairn, R.; Rosman, L. PCBs in the upper and tidal freshwater Hudson River estuary: The science behind the dredging controversy. In *The Hudson River Estuary*; Levinton, J. S., Waldman, J. R., Eds.; Cambridge University Press: New York, NY, 2006; pp 349−367.

(13) USEPA, United States Environmental Protection Agency. *Phase 2 Report - Further Site Characterization and Analysis. Volume 2E - Revised Baseline Ecological Risk Assessment: Hudson River PCBs Reassessment*; USEPA: Region II, NY, 2000.

(14) Bergeron, J. M.; Crews, D.; McLachlan, J. A. PCBs as environmental estrogens: turtle sex determination as a biomarker of environmental contamination. *Environ. Health Perspect.* **1994**, *102*, 780–781.

(15) Cooke, P. S.; Sato, T.; Buchanan, D. L. Disruption of steroid hormone signaling in PCBs. In *PCBs: Recent Advances in Environmental Toxicology and Health Effects*; Robertson, L. W., Hansen, L. G., Eds.; University Press of Kentucky: Lexington, KY, 2001; pp 257−263.

(16) Dickerson, M. S.; Gore, Andrea C. Estrogenic environmental endocrine-disrupting chemical effects on reproductive neuroendocrine function and dysfunction across the life cycle. *Rev. Endocrinol. Metab Disord.* **2007**, *8*, 143–159.

(17) Van Birgelen, A. P.; J., M.; Van der Kolk, J.; Poiger, H.; Van den Berg, M.; Brouwer, A. Interactive effects of 2,2′,4,4′,5,5′-hexachlorbiphenyl and 2,3,7,8-tetrachlorodibenzo-p-dioxin on thyroid hormone, vitamin A, and vitamin B metabolism in the rat. *Chemosphere* **1992**, *25*, 1239–1244.

(18) Morse, D. C.; Koëter, H. B. W. M.; Smits van Prooijen, A. D.; Brouwer, A. Interference of polychlorinated biphenyls in thyroid hormone metabolism: possible neurotoxic consequences in fetal and neonatal rats. *Chemosphere* **1992**, *25*, 165–168.
(19) Morse, D. C.; Groen, D.; Veerman, M.; Van Amerongen, C. J.; Koëter, H. B. W. M.; Smits van Prooijen, A. D.; Visser, T. J.; Koeman, J. H.; Brouwer, A. Interference of polychlorinated biphenyls in hepatic and brain thyroid hormone metabolism in fetal and neonatal rats. *Toxicol. Appl. Pharmacol.* **1993**, *122*, 27–33.
(20) Karasov, W. H. Energetics, physiology, and vertebrate ecology. *Trends. Ecol. Evol.* **1986**, *1*, 101–104.
(21) Rowe, C. L.; Kinney, O. M.; Nagle, R. D.; Congdon, J. D. Elevated maintenance costs in an anuran (Rana catesbeiana) exposed to a mixture of trace elements during the embryonic and early larval periods. *Physiol. Zool.* **1998**, *71*, 27–35.
(22) Lochmiller, R. L.; Deerenberg, C. Trade-offs in evolutionary immunology: just what is the cost of immunity. *Oikos* **2000**, *88*, 87–98.
(23) Congdon, J. D.; Dunham, A. E.; Hopkins, W. A.; Rowe, C. L.; Hinton, T. G. Resource allocation-based life histories: a conceptual basis for studies of ecological toxicology. *Environ. Toxicol. Chem.* **2001**, *20*, 1698–1703.
(24) Dirksen, S.; Boudewijn, T. J.; Slage, L. K.; Mes, R. G.; Vanschaick, M. J. M.; Devoogt, P. Reduced breeding success of cormorants (*Phalacrocorax carbo sinensis*) in relation to persistent organochlorine pollution of aquatic habitats in the Netherlands. *Environ. Pollut.* **1995**, *88*, 119–132.
(25) Ewert, M. A.; Legler, J. M. Hormonal induction of oviposition in turtles. *Herpetologica* **1978**, *34*, 314–318.
(26) Yntema, C. L. Effects of incubation temperature on sexual differentiation in the turtle *Chelydra serpentina*. *J. Morphol.* **1976**, *150*, 453–462.
(27) Rowe, C. L.; Kelly, S. M. Marking hatchling turtles via intraperitoneal placement of PIT tags: Implications for long-term studies. *Herpetol. Rev.* **2005**, *36*, 408–410.
(28) Nagle, R. D.; Rowe, C. L.; Congdon, J. D. Accumulation and selective maternal transfer of contaminants in the turtle *Trachemys scripta* associated with coal ash deposition. *Arch. Environ. Contam. Toxicol.* **2001**, *40*, 531–536.
(29) Becker, P. H.; Schumann, S.; Koepff, C. Hatching failure in common terns (*Sterna hirundo*) in relation to environmental chemicals. *Environ. Pollut.* **1993**, *79*, 207–213.
(30) Brouwer, A.; Ahlborg, U. G.; Van den Berg, M.; Birnbaum, L.; Boersma, E. R.; Bosveld, B.; Denison, M. S.; Gray, L. E.; Hagmar, L.; Holene, E.; Huisman, M.; Jacobson, S. W.; Jacobson, J. L.; Koopman-Esseboom, C.; Koppe, J. G.; Kulig, B. M.; Morse, D. C.; Muckle, G.; Peterson, R. E.; Sauer, P. J. J.; Seegal, R. F.; Prooije, A. E. S. V.; Touwen, B. C. L.; Weisglas-Kuperus, N.; Winneke, G. Functional aspects of developmental toxicity of polyhalogenated aromatic hydrocarbons in experimental animals and human infants. *Eur. J. Pharmacol., Environ. Toxicol. Pharmacol. Sect.* **1995**, *293*, 1–40.
(31) Van Birgelen, A. P. J. M.; Smit, E. A.; Kampen, I. M.; Groeneveld, C. N.; Fase, K. M.; Van der Kolk, J.; Poiger, H.; Van den Berg, M.; Koeman, J. H.; Brouwer, A. Subchronic effects of 2,3,7,8-TCDD or PCBs on thyroid hormone metabolism: use in risk assessment. *Eur. J. Pharmacol., Environ. Toxicol. Pharmacol. Sect.* **1995**, *293*, 77–85.
(32) Hario, M.; Himberg, K.; Hollmén, T.; Rudbäck, E. Polychlorinated biphenyls indiseased lesser black-backed gull (*Larus fuscus fuscus*) chicks from the Gulf of Finland. *Environ. Pollut.* **2000**, *107*, 53–60.

ES9008344