Bull Environ Contam Toxicol (2008) 80:119–122
DOI 10.1007/s00128-007-9328-y

Spatial Variation in Polycyclic Aromatic Hydrocarbon Concentrations in Eggs of Diamondback Terrapins, *Malaclemys terrapin*, from the Patuxent River, Maryland

D. K. Holliday · W. M. Roosenburg · A. A. Elskus

Received: 24 October 2007 / Accepted: 5 December 2007 / Published online: 29 December 2007

Abstract Aquatic organisms encounter a number of contaminants in their environments. Here, we report polycyclic aromatic hydrocarbon (PAH) concentrations detected in diamondback terrapin eggs collected from the Patuxent River, Maryland, one year after an oil spill. Data suggested a geographic difference in egg hydrocarbon concentrations. However, at one year after the oil spill, most PAH concentrations detected were low, were not correlated with the extent of shoreline oiling, and thus likely represent current background levels. Future research should investigate the route of egg PAH exposure and include studies of embryotoxicity.

D. K. Holliday (✉) · W. M. Roosenburg
Department of Biological Sciences, Ohio University, Irvine Hall, Athens, Ohio 45701, USA
e-mail: hollidad@marshall.edu

Present Address:
D. K. Holliday
Department of Anatomy and Pathology, Joan C. Edwards School of Medicine, Marshall University, One John Marshall Drive, Huntington, WV 25755, USA

D. K. Holliday
Department of Integrated Science and Technology, Marshall University, One John Marshall Drive, Huntington, WV 25755, USA

A. A. Elskus
Department of Biology, University of Kentucky, Lexington, KY, USA

Present Address:
A. A. Elskus
United States Geological Survey, Leetown Science Center, S.O. Conte Anadromous Fish Research Laboratory, University of Maine, Maine Field Office, 5751 Murray Hall, Orono, ME 04469, USA

Keywords Polycyclic aromatic hydrocarbons · Turtles · Spatial variation

Aquatic organisms are exposed to a variety of environmental contaminants including heavy metals, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs). For many vertebrates, such as turtles, eggs can be useful indicators of contaminant exposure. Previous research (Bishop et al. 1994; Bonin et al. 1995) found that organochlorine concentrations in snapping turtle eggs reflect the spatial variation of these contaminants within the turtle's habitat. Diamondback terrapin (*Malaclemys terrapin*) eggs may also be useful in elucidating spatial contamination patterns and levels of organismal exposure due to their wide distribution throughout estuaries in the eastern seaboard and the gulf coast of the United States. Terrapins in the Patuxent River, Maryland are locally abundant and show strong site fidelity throughout their lifetime (Roosenburg unpublished), and thus provide a convenient way to assess toxin exposure of particular subpopulations.

Here we present a chemical analysis of Patuxent River terrapin eggs collected 1 year after the Swanson's Creek oil spill. We hypothesized that PAH concentrations would be highest in eggs collected from sites closest to the spill and from areas of heavy shoreline oiling.

Materials and Methods

On April 7, 2000, approximately 140,000 gallons of #6 crude and #2 fuel oil leaked into Swanson's Creek, a tributary of the Patuxent River, Maryland. The oil contaminated approximately 17 miles of shoreline (Collins et al. 2003) including many areas which have been the focus of a 20-year terrapin demographic study by one of the authors (WMR).

Between June 25 and July 1, 2001, approximately 14 months after the spill, we located 12 recently constructed (<24 h old) terrapin nests on beaches along the western (from Golden Beach south to Cremona) and eastern shores (Sheridan point and Craney Creek) of the Patuxent River within the spill area (Fig. 1a). Three eggs from each nest were frozen in solvent-washed glass jars and transported to the University of Kentucky for hydrocarbon analysis. Eggs were pooled by nest, homogenized, and an aliquot used for wet/dry determination. Of the remainder, one gram of homogenate was combined with four grams of sodium sulfate and ground in a mortar. Each sample was spiked with a surrogate standard (50 µL of 8.33 ng/µL *p*-terphenyl) and soxhlet-extracted in 1:1 acetone:hexane (pesticide-grade Burdick and Jackson solvents) for 12 h. Extracts were volume reduced to 1 mL and eluted through glass columns containing 5 g of 5% water-deactivated silica and 2.4 g of 5% water-deactivated alumina to remove interfering lipids. Samples were eluted from the column with 30 mL hexane (discarded) followed by 30 mL of 25% methylene chloride in hexane (PAH fraction), and the PAH fraction concentrated to 0.5 mL and solvent-exchanged to heptane under nitrogen. The PAH fraction was spiked with a recovery standard (50 µL of 8.33 ng/µL *m*-terphenyl), volume reduced to 1 mL and injected (2 µL, splitless) onto a Shimadzu 17A GC-FID fitted with a 30 m DB5 (J& W Scientific) column with hydrogen as the carrier gas. Peak retention times were compared to a 21-compound PAH standard (Ultra Scientific, RI) and identities confirmed by GC/MS. Only samples with recoveries greater than 70% are reported resulting in five western and two eastern nests. Reported concentrations are corrected for recovery. A procedural blank was included with each set of five samples. Sample detection limits were calculated as (Instrument detection limit) × (final volume of sample extract/sample wet weight) and are reported in Table 1.

We used Spearman-Rank correlation to determine the relationship between total PAHs (tPAHs) and extent of shoreline oiling (Hintze 2004). We pooled nests into two groups (east and west) but due to low sample sizes were unable to make statistical comparisons between shores.

Results and Discussion

Total concentrations of polycyclic aromatic hydrocarbons (tPAH) detected in terrapin eggs collected from the Patuxent River appear to be higher on the eastern shore than the western shore (Fig. 1b). The single nest analyzed from Craney Creek, a heavily impacted site directly across the river from the oil spill (Fig. 1a), contained almost five times more tPAHs than the highest tPAH concentration detected from western shore eggs (Table 1). Sheridan Point, the other eastern shore site, is located approximately 3 nautical miles (5.5 km) down river from Craney Creek and was not oiled (Collins et al. 2003); yet Sheridan Point eggs contained more PAH compounds (16 vs. 7) and nearly twice the tPAH concentrations (2178.9 vs. 1202.5 ng/g d.w.) found in Craney Creek eggs (Table 1). Conversely, eggs from western sites contained very few PAHs, a finding somewhat surprising given the extent of the beach oiling in that area (Collins et al. 2003). One western shore egg sample had no detectable hydrocarbons, two exhibited only one PAH (either perylene or benzo(a)pyrene), and in the two remaining samples we detected small amounts of 3,6 dimethylphenanthrene (Table 1).

A number of studies have used contaminants detected in turtle eggs to document geographic variations in pollution (de Solla et al. 2001; Alam and Brim 2000). In the present

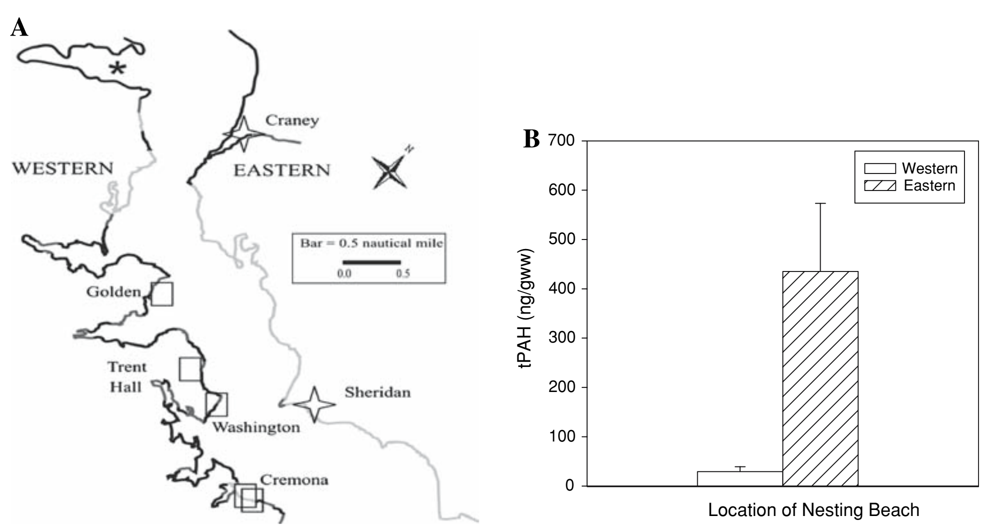

Fig. 1 (**a**) Diamondback terrapin egg collection sites Patuxent River, Maryland. Egg PAH concentrations: squares (<50 ng/g ww), stars (>295 ng/g ww). Shoreline colors represent relative level of oiling (black = moderate, gray = light, light gray = no oil detected; Collins et al. 2003). The asterisk (*) denotes the site of the 2000 oil spill in Swanson's Creek. (**b**) Diamondback terrapin eggs collected on the eastern (n = 2 nests) and western (n = 5 nests) shores of the Patuxent River, Maryland. Bars are means ± 1 standard error

Table 1 PAH residues detected in each clutch from western eggs (Golden, Cremona 1 and 2, Trent Hall and Washington) and eastern eggs (Craney and Sheridan). Values are presented as ng/g wet weight (ww) and dry weight (dw). Detection limits are presented in the first column and a dash (–) denotes values below these limits

PAH	Limit	Golden		Trent Hall		Washington		Cremona1		Cremona2		Craney		Sheridan	
	ww	ww	dw	ww	dw	ww	dw	ww	dw	ww	dw	ww	dw	ww	dw
Naphthalene	2.46	–	–	–	–	–	–	–	–	–	–	–	–	28.0	106.6
Acenaphthylene	2.17	–	–	–	–	–	–	–	–	–	–	–	–	12.4	46.9
Acenaphthene	2.07	–	–	–	–	–	–	–	–	–	–	–	–	13.8	52.4
Fluorene	2.23	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Dibenzthiophene	2.54	–	–	–	–	–	–	–	–	–	–	–	–	12.9	49.1
Phenanthrene	1.87	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Anthracene	1.93	–	–	–	–	–	–	–	–	–	–	21.2	86.9	17.9	67.9
2 Methylphenanthrene	1.94	–	–	–	–	–	–	–	–	–	–	11.7	47.9	28.0	106.2
1 Methylphenanthrene	1.69	–	–	–	–	–	–	–	–	–	–	16.5	67.8	23.8	90.6
3,6 Dimethylphenanthrene	1.60	3.8	11.8	–	–	–	–	–	–	6.7	24.0	76.2	312.5	64.2	244.0
Fluoranthene	1.68	–	–	–	–	–	–	–	–	–	–	54.5	223.5	114.0	433.3
Pyrene	1.68	–	–	–	–	–	–	–	–	–	–	26.4	108.3	23.2	88.1
Benz(a)anthracene	2.09	–	–	–	–	–	–	–	–	2.8	10.0	–	–	15.9	60.6
Chrysene	2.05	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Benzo(b)fluoranthene	3.29	–	–	–	–	–	–	–	–	16.2	58.5	–	–	–	–
Benzo(k)fluoranthene	3.29	–	–	–	–	–	–	–	–	–	–	86.8	355.8	21.6	82.1
Benzo(a)pyrene	6.25	37.6	116.6	–	–	–	–	20.4	85.7	15.3	54.9	–	–	25.1	95.5
Perlyene	4.88	8.5	26.5	4.9	18.1	–	–	–	–	–	–	–	–	29.8	113.2
Indeno(1,2,3-cd) Pyrene	15.15	–	–	–	–	–	–	–	–	–	–	–	–	105.8	402.0
Dibenz(ah)anthracene	17.24	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Benzo(ghi)perylene	20.83	–	–	–	–	–	–	–	–	–	–	–	–	36.9	140.3
tPAH		49.9	154.8	4.9	18.1	–	–	20.4	85.7	41.0	147.4	293.3	1202.5	573.4	2178.9

study, although there appears to be an east–west difference in contaminant concentrations (Fig. 1b), the sample sizes were not large enough to statistically compare tPAHs. Boehm and Farrington (1984) have used a fossil fuel pollution index (FFPI) to determine PAH origin, which could allow us to compare PAH origin among sites. However, this index although useful with sediment concentrations, is unreliable for biological tissues because it does not account for differential uptake of hydrocarbons by the organism or the organism's ability to metabolize the parent compounds.

One potential route of egg PAH exposure is via incubation in contaminated beach sands. The eggs collected in this study were oviposited 14 months after the oil spill and thus, although we did not measure PAHs in substrates, many PAH compounds were likely no longer present in the surface and subsurface nesting sands due to weathering (Wang et al. 2006). Additionally, the extent of shoreline oiling was not correlated ($r = -0.18$) with tPAH concentrations, providing some evidence that egg tPAHs did not originate from nesting beach substrates. Moreover, eggs retrieved from nests in this study had been oviposited no more than 24 h earlier, and thus had limited time in which to absorb contaminants from the substrate. Thus, any substrate-derived contribution is likely to be minor.

A second, more likely, route of exposure is maternal transfer. Lipophilic hydrocarbons can bind to circulating lipid-rich vitellogenin and become incorporated into developing follicles (e.g., Monteverdi and Di Giulio 2000). Sakai et al. (1995) have shown that eggs in the oviduct of adult female sea turtles exhibited high levels of heavy metals deposited during the yolking of follicles. Because complete vitellogenesis can take more than 1 year (Kuchling 1999), terrapins that foraged the previous year in heavily oiled areas may have accumulated PAH concentrations to pass on to their developing follicles. Maternal transfer of lipophilic contaminants via vitellogenin has been reported in fish, including PCBs and PAHs in fathead minnows (Hall and Oris 1991) and PCBs in turtles (Russell et al. 1999). Because like many vertebrates turtles can metabolize organic contaminants (Schlezinger et al. 2000; Yawetz et al. 1998), the lipid-rich follicles likely contain a mixture of parent and metabolite compounds.

Alam and Brim (2000) reported a maximum tPAH of 705 ng/g d.w. in *Caretta caretta* eggs that failed to hatch.

However, the sea turtle egg concentrations were not associated with a specific spill event and thus may represent current, coastal background levels. Because the tPAH concentrations reported in this study from the western nests (maximum tPAH of 147.4 ng/g d.w.) are even lower than those reported in sea turtle eggs, these values likely also represent background levels in the Patuxent. Total PAH concentrations from eastern nests (tPAH 1202.5 and 2178.9 ng/g d.w.) were higher, but still not as high as those reported from other oil spills. Furthermore, there is no correlation between the extent of shoreline oiling and tPAH egg concentration, thus although spatial differences do exist, the tPAHs reported herein may not reflect the 2000 oil spill, but varying concentrations of background signals. Indeed, with the increased industrialization of the Patuxent River drainage, it remains a possibility that PAHs detected in eastern shore eggs were from other, less obvious, fuel oil sources including boating activities and leaching from creosote pilings.

Because we do not know the chromatographic signature of the crude oil spilled into Swanson's Creek, coupled with our small sample size, we cannot establish a link between the 2000 oil spill and the observed tPAH concentrations. Instead, we conclude that many of the hydrocarbons detected were likely due to background PAHs in the environment and that these hydrocarbons are likely being maternally transferred to developing young. Future studies must include chemical analysis of livers, fat stores, and developing follicles from adult female turtles and of nesting beach sediments in order to demonstrate the route of PAH transfer to oviposited eggs and should also include studies of embyrotoxicity (e.g., Van Meter et al. 2006).

Acknowledgements The authors thank the Department of Biology at the University of Kentucky for their support and the Department of Biological Sciences at Ohio University for funding. Thanks to P. Allman, T. Radzio, K. Kelley, and undergraduates for their assistance in the field. Earlier versions of the manuscript greatly benefited from the comments of three anonymous reviewers. Eggs were collected under Scientific Collecting Permit #200160 issued to WMR from the MD-DNR Tidewater Fisheries Division.

References

Alam SK, Brim MS (2000) Organochlorine, PCB, PAH, and metal concentrations in eggs of loggerhead sea turtles (*Caretta caretta*) from northwest Florida, USA. J Environ Sci Health B35:705–724

Bishop CA, Brown GP, Brooks RJ, Lean DRS, Carey JH (1994) Organochlorine contaminant concentrations in eggs and their relationship to body size and clutch characteristics of the female common snapping turtle (*Chelydra serpentina serpentina*) in Lake Ontario, Canada. Arch Environ Con Tox 27:82–87

Boehm PD, Farrington JW (1984) Aspects of the polycyclic aromatic hydrocarbon geochemistry of recent sediments in the Georges Bank Region. Environ Sci Technol 18:840–845

Bonin J, Des Granges JL, Bishop CA, Rodrigue J, Gendron A, Elliott JE (1995) Comparative study of contaminants in the mudpuppy (*Amphibia*) and the common snapping turtle (*Reptilia*), St. Lawrence River, Canada. Arch Environ Con Tox 28:184–194

Collins J, Carlson D, Hilaski S (2003) Joint Information Center Swanson Creek Bulletin, Final Edition

de Solla SR, Bishop CA, Lickers H, Jock K (2001) Organochlorine pesticides, PCBs, Dibenzodioxin, and furan concentrations in common snapping turtle eggs (*Chelydra serpentina serpentina*) in Akwesasne, Mohawk Territory, Ontario, Canada. Arch Environ Con Tox 40:410–417

Hall AT, Oris JT (1991) Anthracene reduces reproductive potential and is maternally transferred during long-term exposure in fathead minnows. Aquatic Toxicol 19:249–264

Hintze J (2004) Number Cruncher Statistical Software (NCSS). Kaysville, Utah

Kuchling G (1999) The reproductive biology of the Chelonia. Springer-Verlag, Berlin

Monteverdi GH, Di Giulio RT (2000) In vitro and in vivo association of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and benzo[a]pyrene with the yolk precursor protein vitellogenin. Environ Toxicol Chem 19:2502–2511

Russell RW, Gobas FAPC, Haffner CD (1999) Maternal transfer and *in ovo* exposure of organochlorines in oviparous organisms: a model and field verification. Environ Sci Technol 33:416–420

Sakai H, Ichihashi H, Saganuma H, Tatsukawa R (1995) Heavy metal monitoring in sea turtles using eggs. Mar Pollut Bull 30:347–353

Schlezinger JJ, Keller J, Verbugge LA, Stegeman JJ. 2000. 3,3′,4,4′-Tetrachlorobiphenyl oxidation in fish, bird and reptile species: relationship to cytochrome P4501A inactivation and reactive oxygen production. Comp Biochem Phys C 125:273–286

Van Meter RJ, Spotila JR, Avery HW. 2006. Polycyclic aromatic hydrocarbons affect survival and development of common snapping turtle (*Chelydra serpentina*) embryos and hatchlings. Environ Pollut 142(3):466–475

Wang ZD, Stout SA, Fingas M. 2006. Forensic fingerprinting of biomarkers for oil spill characterization and source identification. Environ Forensics 7:105–146

Yawetz A, Woodin BR, Stegeman JJ. 1998. Cytochromes P450 (CYP) in liver of the turtle *Chrysemys picta picta* and the induction and partial purification of CYP1A-like proteins. Biochim Biophys Acta 1381:12–26