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Diamondback terrapins as indicator species of persistent organic pollutants: Using Barnegat Bay, New Jersey as a case study

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ABSTRACT

The diamondback terrapin's (*Malaclemys terrapin*) wide geographic distribution, long life span, occurrence in a variety of habitats within the saltmarsh ecosystem, predatory foraging behavior, and high site fidelity make it a useful indicator species for contaminant monitoring in estuarine ecosystems. In this study fat biopsies and plasma samples were collected from males and females from two sites within Barnegat Bay, New Jersey, as well as tissues from a gravid female and blue mussels (*Mytilus edulis*), which are terrapin prey. Samples were analyzed for persistent organic pollutants (POPs), including polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), chlorinated pesticides, and methyl-triclosan. Terrapins from the northern site, Spizzle Creek, closest to influences from industrial areas, had higher POP concentrations for both tissues than terrapins from the less impacted Forsythe National Wildlife Refuge. Sex differences were observed with males having higher contaminant concentrations in fat and females in plasma. PCB patterns in terrapin fat and plasma were comparable to other wildlife. An atypical PBDE pattern was observed, dominated by PBDEs 153 and 100 instead of PBDEs 47 and 99, which has been documented in only a few other turtle species. The typical PBDE patterns measured in mussels, terrapin prey, suggests that the terrapin may efficiently biotransform or eliminate PBDE 47 and possibly PBDE 99. Plasma contaminant concentrations significantly and positively correlated with those in fat. This study addresses several aspects of using the terrapin as an indicator species for POP monitoring: site and sex differences, tissue sampling choices, maternal transfer, and biomagnification.

1. Introduction

Polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and organochlorine pesticides (OCPs) are persistent organic pollutants (POPs). Their lipophilicity results in biomagnification, which can increase the possibility of toxic effects at higher trophic levels. PCBs used in electrical components, plastics and adhesives, and OCPs, such as oxychlordane, dieldrin, mirex and DDT, including their associated metabolites, are often referred to as legacy compounds because their use in several countries has been banned or restricted for decades, but they still persist in the environment (Eisler, 1986). PBDEs are additive flame retardants commonly found in many current-use products, such as televisions and furniture foams. Their use is becoming restricted because they have been detected in wildlife and humans globally and have toxic properties (Darnerud et al., 2001; Hites, 2004). Toxic effects of PCBs, PBDEs and OCPs include disrupted neurobehavioral development, reduced reproductive success, carcinogenicity and endocrine effects (de Solla et al., 1998; Darnerud et al., 2001; Kulkarni, 2005). These toxic effects may impact wildlife populations and be especially detrimental to already threatened and/or endangered species.

POP concentrations are often monitored in the environment using indicator species. Many reptiles possess characteristics that make them useful indicators, such as a long life span, wide geographic range, use of a variety of habitats, high trophic position, and site fidelity (Blanvillain et al., 2007). Turtles have been used extensively to monitor POPs, especially in the Great Lakes region. For example, the snapping turtle (*Chelydra serpentina*) is commonly used to monitor the organic contamination in the Great Lakes (Bishop et al., 1996; Bishop et al., 1998; de Solla et al., 2007). Concentrations of PCBs measured in snapping turtle eggs correlated with the industrial use of specific technical mixtures in the region (de Solla et al., 2007). In fact, the diamondback terrapin (*Malaclemys terrapin*) has been implied as a suitable indicator of both POP and mercury contamination in estuarine ecosystems (Kannan et al., 1998; Blanvillain et al., 2007). Turtles can also be used as indicators of toxic effects. For example, temperature-dependent sex determination in the red-eared slider (*Trachemys*

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scripta) is disturbed with estrogenic PCB exposure by reversing gonadal sex (Bergeron et al., 1994). This type of endocrine disruption has the potential to alter sex ratios and affect future reproduction of individuals.

The diamondback terrapin has characteristics that make it a good indicator species for estuarine POP contamination (Blanvillain et al., 2007). The terrapin geographic distribution spans estuaries along most of the eastern coast of the US and throughout the Gulf of Mexico. Terrapins are relatively long-lived, with a life span of 30 years or more (Brennessel, 2006). They utilize a variety of estuarine habitats, including saltmarshes, beaches and open bays. Terrapins show site fidelity to specific creeks and rivers, and females have nest site fidelity through many nesting seasons (Roosenburg, 1994; Gibbons et al., 2001). Their diet consists mainly of fish, crabs, mussels, clams, shrimp and snails. On occasion they feed on carrion and ingest barnacles, algae, grass and mud as a consequence of consuming their prey (Brennessel, 2006). Of the seven sub-species of diamondback terrapins, the northern diamondback terrapin is listed as a species of special concern (NJDEP, 2008). They are subjected to many anthropogenic impacts because their habitat is of great economic and recreational importance to humans. Habitat fragmentation and degradation, drowning in crab pots and injuries by boats and cars are the most documented and tangible threats (Gibbons et al., 2001). Exposure to anthropogenic toxic chemicals is also a possible threat to terrapin populations, but one that is far less noticeable or studied.

The objective of this study was to determine the occurrence of POPs in terrapins in Barnegat Bay, New Jersey thereby assessing the utility of the terrapin as an indicator species of POP contamination in an estuary with varied human uses. Two sites were chosen to represent different levels of human influence, and POP concentrations were measured in both blood and fat biopsies of live terrapins. In this way, site differences could infer the usefulness of the terrapin as an indicator species and a comparison of paired blood and fat samples could provide information about the most efficient and least invasive sampling method for measuring POPs in terrapins. Secondary objectives, most of which are presented in the main text but are further discussed in Supplementary materials, included assessing POP distributions among several terrapin tissues and eggs, comparing POP concentrations between sexes to investigate maternal transfer and calculating preliminary biomagnifications factors using terrapin prey, the blue mussel (*Mytilus edulis*).

2. Materials and methods

2.1. Study site and sample collection

In September 2006, 16 diamondback terrapins were captured in Barnegat Bay, New Jersey half male and half female. Trapping was concentrated at two sites of the Bay. The southern site is located in the Edwin B. Forsythe National Wildlife Refuge, Barnegat Division, on the mainland side of the bay (Forsythe) (Fig. 1). The northern site, Spizzle Creek is located in Island Beach State Park on the barrier island side of the bay (Spizzle) (Fig. 1). Spizzle is north of Forsythe by about a 10 km straight-line distance, across the width of the bay. The northern portion of Barnegat Bay is the most industrialized and densely populated and also collects 70% of the inflow of water from the watershed to the bay. Thus the Spizzle site is more likely affected by urban and industrial contaminants in the watershed. A blood sample and fat biopsy was taken from each terrapin. Various tissues, including 11 eggs, were collected from one additional deceased turtle from Forsythe as well as 19 blue mussels from Forsythe. The deceased turtle was a trap mortality and was in excellent health. Terrapin trapping, handling and sample collection and storage techniques are described in Supplementary materials.

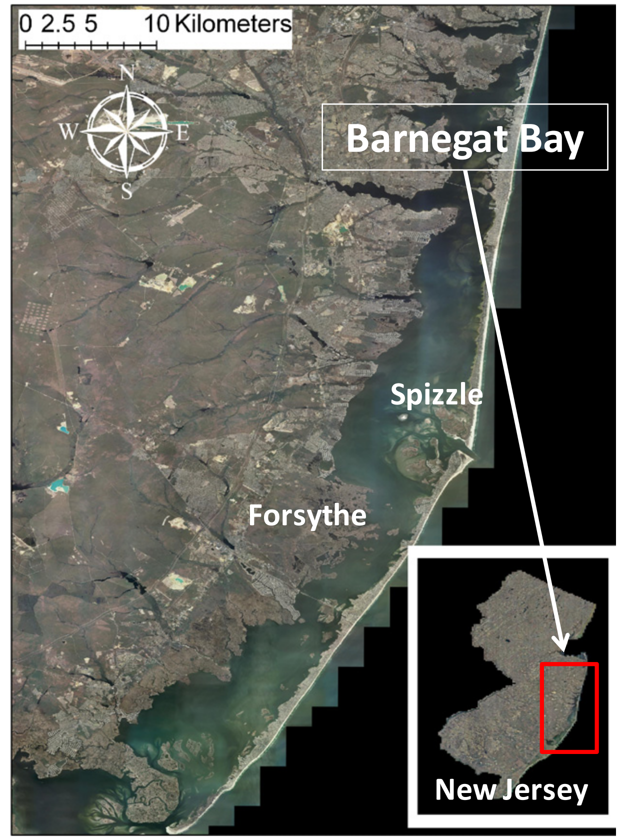

Fig. 1. Map of Barnegat Bay, New Jersey. Overview of bay depicting two sampling locations. Northern site Spizzle is about 10 km straight-line distance from southern site Forsythe.

2.2. Calibration solutions and quality control

Calibration solutions were prepared by combining National Institute of Standards and Technology (NIST) Standard Reference Materials (SRMs): 2261 Chlorinated Pesticides in Hexane, 2275 Chlorinated Pesticides in Hexane II, 2262 Chlorinated Biphenyl Congeners in 2,2,4-Trimethylpentane, 2274 PCB Congener Solution II, and solutions containing an additional 46 PCB congeners and 27 PBDE congeners. These were used to prepare seven calibration solutions at differing concentrations that were extracted and cleaned up alongside samples. Internal standard solutions, added to samples prior to extraction, contained ^{13}C-labeled PCB congeners (28, 52, 77, 126, 169, 118, 153, 180, 194, and 206), 6-F-PBDE 47, PBDE 104, 4′-F-PBDE 160, 4′-F-PBDE 208, ^{13}C-labeled PBDE 209, ^{13}C-labeled pesticides (HCB, *trans*-chlordane, *trans*-nonachlor, oxychlordane, dieldrin, 4,4′-DDE, 4,4′-DDT), 4,4′-DDD-d_8, and ^{13}C-labeled methyl-triclosan.

Two NIST SRMs were used as control samples. One replicate of SRM 1589a (PCBs, Pesticides, PBDEs, and Dioxins/Furans in Human Serum) in addition to one replicate of an in-house control material of pooled loggerhead plasma (Cc pool) were analyzed with the plasma samples. Two replicates of SRM 1946 (Lake Superior Fish Tissue) were analyzed with the fat samples. Laboratory procedural blanks and blanks made from each lot of blood collection supplies were also analyzed.

2.3. Sample analysis

Plasma from the 16 live terrapins and the blood from the necropsied female (≈2 g each) were equilibrated with an ethanol