Persistent Organic Pollutants in Diamondback Terrapin (*Malaclemys terrapin*) Tissues and Eggs, and Sediments in Barnegat Bay, New Jersey

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ABSTRACT

Persistent Organic Pollutants in Diamondback Terrapin (*Malaclemys terrapin*) Tissues and Eggs, and Sediments in Barnegat Bay, New Jersey

Emily R. Basile

Persistent organic pollutants (POPs) are ubiquitous in the environment and have the potential to become a health risk to wildlife by eliciting toxic effects and altering survival and reproduction. The group of studies included in this dissertation characterizes POP contamination levels and patterns in an estuary located on the central coast of New Jersey by utilizing a model estuarine vertebrate, the diamondback terrapin (*Malaclemys terrapin*). I determined that the terrapin is a suitable bioindicator of organic contamination in an estuarine environment and can be used as indicators of local contamination by collecting a non-lethal plasma sample which represents the stored contaminant burden. The POP concentrations and patterns in the terrapin tissues collected suggest that Barnegat Bay, New Jersey has relevant levels of organic contaminants relative to other wildlife species, with the exception of a contaminant class of emerging concern, polybrominated diphenyl ethers (PBDEs). Terrapin tissues and Barnegat Bay nesting beach sediment revealed an atypical PBDE pattern with a higher prevalence of hexa-brominated PBDEs (154, 153 and 100) instead of the normal predominance of lower tetra and penta-brominated PBDEs (47 and 99). The data in these studies indicate maternal transfer of all POPs reported and that it is the major source of POPs to developing terrapin embryos. In an experiment where terrapin eggs were incubated in sediments spiked with PBDEs, transfer of PBDEs and other POPs from

nesting sediment into developing eggs was found to be negligible. Therefore transfer of POPs from natural nesting sediments into eggs is unsubstantial unless that natural sediment is highly contaminated. Examination of three health endpoints in terrapins suggest that environmentally relevant concentrations of mirex, PCBs and PBDEs may be associated with immune and endocrine disruption and that PBDE 47 may be associated with a disruption in neurobehavioral development. These data suggest that terrapins may also be useful as bioindicators of endocrine disruption and immunotoxicity within the estuarine environment in respect to other species and humans.

CHAPTER 1: INTRODUCTION

Persistent Organic Pollutants

Persistent organic pollutants, commonly referred to as POPs, are a group of chemicals that share four specific characteristics. They are persistent in the environment, lipophilic, bioaccumulative and are capable of long range environmental transport (Jones and de Voogt, 1999). Their presence in the environment is persistent because these chemicals typically have long half lives and are resistant to degradation by environmental processes. POPs are lipophilic, meaning fat-loving/ water-hating chemicals. They will partition into animal tissues very easily. Because POPs partition into animal tissues, they biomagnify as they pass through the trophic levels and can accumulate in top predator tissues to levels that can cause toxic effects (Jones and de Voogt, 1999). In the environment they partition into soils and sediments, generally to the organic matter associated with the substrate. Due to the relatively low vapor pressures associated with these classes of chemicals, POPs volatilize into the air in warmer temperatures. The POPs can either become associated with particulate matter in the air or exist in a gaseous phase (Bidleman, 1988). Their existence in either phase, gas or particulate, depends on certain factors such as vapor pressure of the POP, the ambient temperature and the concentration and size of particulate matter in the air (Bidleman, 1988). Once POPs are associated with the air they can then be deposited back into the environment through wet or dry deposition which is

often associated with cooler temperatures and air particle sizes. Global distillation is the process where POPs volatilize from tropic and temperate areas and travel in the air over land and/ or oceans to polar regions (Bidleman, 1988). This process can move POPs to places in the world such as the Arctic and put indigenous people and wildlife who had no part in the manufacturing or use of these chemicals at risk for toxic effects (Jones and de Voogt, 1999).

In May of 2001, the Governing Council of the United Nations Environment Programme (UNEP) presented the Stockholm Convention for Persistent Organic Pollutants (Stockholm Convention). This international treaty was constructed over five years of planning and negotiating. To date, over 160 countries have signed and ratified the Stockholm Convention, the United State not being one of them (Programme, 2009). The document was created with the intention to protect human health and the environment from the toxic effects of POPs. The convention's goals include obligations to those countries that have signed and ratified it to eliminate and/or restrict the production and use of the designated POPs in the convention. Another was to prevent and control the release of POPs that are created as a by-product of combustion and other processes as well as the safe and proper disposal and/or destruction of POPs when they become waste (Lallas, 2001). When the Stockholm Convention was opened in 2001 there were 12 POPs included: aldrin, chlordane, dieldrin, heptachlor, mirex, toxaphene, DDT, hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs), dioxins and furans (Programme, 2009). In 2009 the convention was

amended and nine new POPs were added: chlordecone, hexabromobiphenyl, lindane, alpha hexachlorocyclohexane, beta hexachlorocyclohexane, tetra thru heptabromodiphenyl ethers (PBDEs), perflorooctane sulfonic acid (PFOS) and pentachlorobenzene (Programme, 2009). For the purpose of the following studies in this dissertation I will only elaborate on the POPs that were measured and analyzed in the samples collected.

Polychlorinated biphenyls (PCBs)

Polychlorinated biphenyls (PCBs) are a class of compounds that began commercial production in 1929 and were used in hydraulic fluids, heat transfer fluids and high pressure lubricants, as additives to paint, ink dies, plasticizers, protective coatings for wood, dedusting agents, adhesives, pesticide extenders, ink and dye carrier, and microencapsulation for dyes for carbonless duplicating paper (George et al., 1988). Production of PCBs was ceased in 1977 but the effects are still present in the environment. There are theoretically 209 possible congeners of PCBs based on the amount of chlorination on the biphenyl rings (Brown et al., 2004). Some congeners are more environmentally relevant and have been thoroughly investigated for possible toxic effects. The toxic effects include endocrine disruption, immunotoxicity, developmental toxicity, reproductive toxicity and carcinogenicity (ATSDR, 2000). Specifically, congeners PCB 126, 118, 52 and 153 have shown dioxin like toxicity, and have even been reported to be more potent then TCDD itself (Ahlborg et al., 1994).

Organochlorine pesticides (OCPs) are a group of chemical whose primary use is for pest control and all have chlorine in their chemical structures. Many of these compounds are legacy compounds, those that are no longer in use today, but because of their persistence in the environment are still measured in biotic and abiotic samples.

Mirex

Mirex, which is no longer used in the United Sates, was a compound used to both control fire ants and as a flame retardant in plastic, rubber, paint and electrical goods (Brown et al., 2004). It was manufactured and used in the 1960's and 1970's and contaminated water and soil where they were being manufactured and used (ATSDR, 1995).

Dichlorodiphenyltrichloroethane (DDT)

DDT was used as an insecticide to control pests on agricultural crops as well as to control typhus and malaria vectors. It is no longer used in the United States but is still used in some countries for malaria control. The use of DDT was ceased in the United States in 1972 and the production for export was ceased after 1985. The parent compound DDT entered air, water and soil during its production and use. Its metabolites include isomers of DDE and DDD. In most biological samples as well as in the current studies the most common metabolite measured in terrapins tissues was p,p'dichlorodiphenyltrichloroethane (4,4'-DDE) and is the compound generally associated with toxological effects in organisms (ATSDR, 2002b). This is because this metabolite is the most stable in the

environment. When the parent compound is measured in samples it is a sign of recent use and exposure to the chemical where as DDE represents persistence and historical contamination.

Dieldrin

Dieldrin was an insecticide used to control pests on crops such as corn and cotton as well as to kill termites in homes. It was used from the 1950's to 1970's until the Department of Agriculture cancelled its use, but in 1972 the EPA allowed the use of dieldrin for termite control in homes until the manufacturer cancelled the registration voluntarily in 1987. This compound can be found in air, surface water, groundwater, soil and sediment (ATSDR, 2002a).

Hexachlorobenzene (HCB)

Hexachlorobenzene (HCB) was used as a fungicide on the seeds of onions, sorghum, wheat, and other grains until 1984. It was also used in the production of pyrotechnic and ordinance materials for the military, the production of synthetic rubber, as a porosity controller in the manufacture of electrodes, a chemical intermediate in dye manufacturing, and a wood preservative. HCB is also produced as a by-product during the production of many other chlorinated chemicals therefore it is still produced. HCB can enter the environment through air emissions as well as waste water from facilities that produce it as a by-product. It is also introduced in the environment because it in an impurity associated with several widely used pesticides (ATSDR, 2002c).

Oxychlordane

Oxychlordane is a residue created through metabolism of chlordane which was a pesticide used to control termites in homes in the United States from 1948 to 1988. Its application was often underground around the foundation of homes and soil around homes to kill termites. Before 1978 it was also used as a pesticide for crops, lawn and gardens. All use in the United States was ceased in 1988 but manufacture for export still exists. Chlordane can enter the environment through the air and sorb to sediments (ATSDR, 1994).

All of the OCPs discussed are man-made and do not occur naturally in the environment therefore any existence of POPs in abiotic and biotic samples is anthropogenic in nature. They strongly associate with sediments and soils and are capable of volatilization into the air and long range atmospheric transport to locations where they are not used or produced. Toxic effects associated with these OCPs include carcinogenicity, developmental and reproductive toxicity, endocrine disruption and immunotoxicity (ATSDR, 1994, 1995; Jones and de Voogt, 1999; ATSDR, 2002a, c, b).

Polybrominated diphenyl ethers (PBDEs)

Polybrominated diphenyl ethers (PBDEs) are a family of chemicals used as additive flame retardants. Production of PBDEs for commercial use began in the 1970's (ATSDR, 2004). Deca-BDE is still manufactured and used in the United States today but the penta and octa mixtures have been banned (Klosterhaus et al., 2008). PBDEs are used and found in plastics, building

materials, and textile coatings (including clothing) such as ready-made plastic products, acrylonitrile butadiene styrene and high impact polystyrene as well as electronic devices, such as cabinets and circuit boards in personal computers, television sets, electrical cables switches and capacitors (Darnerud et al., 2001). Although PBDEs are man-made for commercial use, hydroxylated and methoxylated PBDEs as well as polybrominated dibenzo-p-dioxins have been discovered as natural compounds in marine species (Malmvarn et al., 2008). Hydroxylated PBDEs and polybrominated dibenzo-p-dioxins also occur in environmental samples by anthropogenic inputs (Malmvarn et al., 2008). Theoretically there are 209 possible congeners of PBDEs similar to PCBs. The lower brominated compounds (tetra and penta-BDEs) are the most bioavailable and therefore are the most accumulative in animal tissue (Hites, 2004). The higher brominated compounds like deca-BDE (PBDE 209), which are the only ones still in production, have been documented to debrominate by anaerobic, photolytic and microbial reductive pathways to the lower brominated and more toxic compounds (Soderstrom et al., 2004; Gerecke et al., 2005; He et al., 2006). Higher and lower brominated compounds have also been reported to debrominate through biotransformation in the common carp (*Cyprinus carpio*) (Stapleton et al., 2004a,b). Toxic effects reported for PBDEs include endocrine disruption, developmental neurotoxicity, and possible reduced reproductive success and carcinogenicity (Fowles et al., 1994; Eriksson et al., 1999; Darnerud et al., 2001; Fernie et al., 2005; Fernie et al., 2008). Sources of PBDEs into the environment

include release during the initial synthesis of the chemicals, the incorporation into the product, the use of the product and then the disposal of the product (Hale et al., 2003).

Although these contaminants are persistent in the environment they will decline over time if production and use of products with POPs decrease. There are many studies on the temporal trends of POPs in many different media, both abiotic and biotic. Both sediment samples which are representative of the atmospheric deposition of POPs and breast milk samples which are representative of the accumulation in higher trophic level organisms are showing decreasing trends for PCBs and OCPs. PBDEs however, are still increasing (Hites, 2004; Covaci et al., 2005; Lignell et al., 2009).

Barnegat Bay, New Jersey

Barnegat Bay is a shallow lagoon type estuary stretching about 70 km along the coastline of central New Jersey. The barrier islands sheltering the bay provide an almost continuous border. To the north is the barrier island called Island Beach which is a state park. South of Barnegat Inlet is Long Beach Island. The watershed houses a population of around 500,000 but that number more than doubles during summer months. The watershed covers about 1732 km^2 of land and is almost completely contained in one county, Ocean County, although it extends into parts of Monmouth and Burlington counties (BBNEP, 2001). Human land use dominates about 28% of that land. The largest tributaries that empty into the bay are north of Barnegat Inlet and are responsible for draining

70% of the watershed. Toms River is one of the most important influent systems in the northern region of the bay and is responsible for 31% of the surface water that enters the bay. Barnegat bay is not only shallow, only about 1-7 meters deep, but it is also relatively slow moving, with a turnover rate of about 27-71 days. The Bay's circulation is driven mostly by coastal pumping and remains fairly consistent (BBNEP, 2001).

Estuaries are essential environments because they are critical habitats for diverse wildlife and are one of the most productive ecosystems on the planet. Not only are they critical habitats for wildlife, they are also an important habitat for humans and their associated impacts. In 1995, under an amendment to the Clean Water Act, the Environmental Protection Agency (EPA) enlisted Barnegat Bay into the National Estuary Program where grant money is provided to manage estuaries threatened by pollution, land development and overuse. There are currently 28 estuaries in the United States receiving funding through this program. The primary management issues in Barnegat Bay include nutrients, conventional pollutants (basically those which are treated or found in waste water), pathogens, human population growth, habitat loss/ alteration, species loss/ decline, fisheries loss/decline, introduced/pest species, freshwater inflow and drinking water problems (USEPA, 2010).

Although there are many management issues listed above, the EPA describes non-point source pollution as being the most important issue when addressing the issues surrounding water quality and the health of the living

resources of the Bay (USEPA, 2010). There are many anthropogenic inputs that can affect water quality. However, for the purpose of these studies the focus will be on the non-point source pollution of Barnegat Bay by persistent organic pollutants. In Barnegat Bay's management plan there are few programs proposed and even fewer that actually have been implemented, that include the monitoring of organic pollutants. One is the Ambient Water Monitoring Network which has five stations within the Barnegat Bay watershed that are sampled four times a year. Organic pesticides, volatile organic contaminants and bed sediment contaminants are part of those analyses but unfortunately those three parameters are on a reduced sampling frequency. Another is the Environmental Monitoring and Assessment Program (EMAP) which is a national program funded and organized by the EPA. This program samples several locations within the Barnegat Bay region, however locations are not fixed (BBEP et al., 2002). Estuarine sediment is a major sink for organic contaminants in the aquatic environment. The slow movement of water and the occurrence of fine grained sediment allow an adsorptive capability of these contaminants to the sediments (Voorspoels et al., 2004). Because these contaminants are strongly associated with the sediment, they are available for interaction and can become a source of POPs to the benthic community. The benthic organisms are often the base of many food chains in an estuary. POPs can be monitored through abiotic sampling of estuarine sediments, but a better representation of POP contamination and its

possible effects to estuarine health and function is better examined through higher tropic level organisms.

The most probable non-point sources of organic pollutants in Barnegat Bay are atmospheric deposition, waste water treatment, landfills and underground storage and septic systems. Atmospheric deposition of POPs is most likely the greatest non-point source contributing to the contamination of Barnegat Bay sediments and water. As discussed previously POPs can volatilize and become associated with the air, then travel and deposit back into sediments, soils and water (Bidleman, 1988). Prior to 1980, waste water from treatment facilities was discharged to streams, rivers, the estuary or fields in the watershed (BBNEP, 2001). POPs are not intentionally removed during any of the treatment levels in WWTP. They will sorb to the sludge and solids that are eventually applied back into the environment through disposal or land application for agriculture. They can also be associated with any suspended solid in effluents therefore they enter the aquatic ecosystem directly (Katsoyiannis and Samara, 2005). In 1970 the Ocean County Utilities Authority (OCUA) was created to control and regulate the treatment of waste waters. Prior to the organization of OCUA, there were 46 small to medium treatment facilities run by municipalities, developers and private sewer companies. Now there are only three OCUA Regional Wastewater Treatment Facilities, and the effluents are transported by outfall lines to the ocean that eventually mix the effluent with ambient ocean water (BBNEP, 2001). Septic systems, underground storage tanks and landfills are all possible sources of non-

point source POP pollution. The POPs associated with these systems are able to enter the groundwater very easily and quickly because most of the Barnegat Bay watershed is composed of Cohansey sands which do not absorb or breakdown contaminants (BBNEP, 2001). Underground storage tanks that house gas and oil have the potential to leak. Tanks under 2000 gallons are not required by law to be checked for leaks and often are not checked. Landfills in the past were constructed without liners or caps so rainwater percolates through the garbage, and the lechate is able to contaminate groundwater (BBNEP, 2001).

Diamondback Terrapins

The diamondback terrapin (*Malaclemys terrapin*) is a mid-sized strictly estuarine emydid turtle. There are seven sub species of the terrapin that stretch from New England into the Gulf of Mexico (Brennessel, 2006). The species inhabiting Barnegat Bay New Jersey is called the Northern Diamondback Terrapin (*Malaclemys terrapin terrapin*) and is listed as a species of special concern (Mohrman, 2009). Terrapins are a relatively long-lived species, with a life span of 30 years or more (Roosenburg and Kelley, 1990). Adult terrapins exhibit sexual dimorphism in respect to their size where females are often more than twice the size of an adult male. Diets of terrapins consist 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). They utilize a variety of estuarine habitats, including the saltmarshes, beaches and open bay. Terrapins show site fidelity to specific creeks

and rivers, and females have nest site fidelity through many nesting seasons (Burger, 1977; Roosenburg, 1996; Gibbons JW, 2001; Mitro, 2003). Terrapins are active in the warmer months, from about April to October-November. In Barnegat Bay they nest during the months of June and July and can lay multiple clutches in one season. Their clutches range from 6-18 eggs and are incubated in sandy substrates. Once the weather becomes cooler terrapins will brumate in the benthic sediments and respire through cloacal respiration (Brennessel, 2006). Species that are habitat generalists with a long life span, a wide geographic distribution, that exist in a variety of habitats, have a high trophic position and exhibit site fidelity are excellent species to use as bioindicators (Blanvillain et al., 2007). Many reptiles fit this list of characteristics including turtles (Meyers-Schone, 1994). 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). Turtles can also be used as indicators of toxic effects (Meyers-Schone, 1994). Because of the terrapins long life span, wide geographic distribution, occurrence in a variety of habitats within the saltmarsh ecosystem, predatory foraging behavior, and high site fidelity it is an excellent indicator species for contaminant monitoring in the estuarine ecosystem. The terrapin has already been reported as a suitable indicator of contamination in an estuarine ecosystem (Kannan et al., 1998; Blanvillain et al., 2007).

There are many possible route of exposure of POPs to a terrapin through all stages of life. Typical routes of exposure include dermal exposure, inhilation and ingestion of the compounds. Dermal exposure of POPs to terrapins may occur through their skin when in contact with contaminated water, sediments and possibly air when basking on land. Hatchling, juvenille and adult terrapins would be more likely exposed to contamiants through contact with water but terrapin eggs may be exposed through contact by incubating in contamined nesting sediments. Inhilation of POPs may occur because terrapins are air breathing species who respire at the air:sea surface where POPs are known to accumulate by atmosphereic deposition (BrorstromLunden, 1996). Any contamiants associated with the air could potentilay provide a source to terrapins at all stages of life. Both dermal exposure and inhilation of POPs most likely contribute a negligable amount of contaminats to terrapins. Ingestion of POPs is assumed to be the major route of exposure to juvenile and adult terrapins from which they most likely accumulate the majority of their adult contaminant burden. Because they inhabit a relatively high trophic level, they are subject to the biomagnification of POPs through the food chain. They may also ingest contaminated water and sediments that may contribute to the accumulation of POPs in adults over time. There are two other unique route of exposure of POPs to terrapins. The first is maternal transfer of POPs from the female to her offspring. Portions of the maternal contaminant burdens have been reported to be incorporated with the yolk in an egg making it available to the developing embryo (Russell et al., 1999). This is

most likely the major source of POPs to developing terrapin embryos and hatchling s. The second is through uptake from substrate during brumation. Because turtles take up oxygen from the water through cloacal respiration, contaminants associated with the water or particles in the water may pass into terrapin tissues (Bell 2006).

The main goals from this group of studies discussed in this dissertation were to describe the contamination of Barnegat Bay, New Jersey by persistent organic pollutants using a model estuarine vertebrate and to provide possible health threats associated with the environmental levels measured. The main objectives of chapters two and three were to report the contamination profiles of POPs in terrapin tissues, eggs and nesting beach sediments. The fourth chapter introduces data on the ability of POPs, specifically PBDEs, to transfer from nesting sediments into developing terrapin eggs. The fifth chapter reports possible health endpoints associated with environmentally relevant concentrations of POPs found in terrapin plasma and eggs. The final chapter will conclude the findings from the studies conducted and discuss future implications and actions that should be taken in light of the data collected and conclusions drawn.

CHAPTER 2:
DIAMONDBACK TERRAPINS AS INDICATOR SPECIES OF PERSISTENT ORGANIC POLLUTANTS: USING BARNEGAT BAY, NEW JERSEY AS A CASE STUDY

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.

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). Diamondback terrapins have been implied as suitable indicators of PCB 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 scripta*) is disturbed by estrogenic PCB exposure (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 (*Malaclemys 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 U.S. 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 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)*.

Materials and Methods

Study Site and Field Collection

In September 2006, 16 diamondback terrapins were captured in Barnegat Bay, New Jersey. 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). The northern site at Spizzle Creek in Island Beach State Park is located on the barrier island side of the bay (Spizzle). 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 industrial contaminants in the watershed.

Terrapins were trapped using hoop traps and fyke nets set in marsh creeks. Ten individuals, five females (884-1659 g) and five males (364-422 g) were captured from Forsythe. Six individuals, three females (649-1191 g) and three

males (313 -454 g) were captured from Spizzle. All terrapins were weighed, measured, and an identification code was notched into the scutes. Most females and larger males were also given passive integrated transponder (PIT) tags for identification. Terrapins were transported to laboratories and tissue collection occurred within 12-24 h. Terrapin handling and sampling was performed in accordance with an approved animal care and use protocol from Drexel University.

Tissue Collection

Blood was drawn using 21 gauge double-ended needles into 2 mL glass Vacutainer tubes coated with sodium heparin (Becton, Dickinson and Company, Franklin Lakes, NJ). The subcarapacial venipuncture method was used for blood collection as described by Hernandez-Divers et al. (Hernandez-Divers, 2002) and adapted by other terrapin researchers (D.W. Owens, College of Charleston, Charleston, SC, USA, personal communication). Generally, 4-6 mL of blood was drawn from females and 3-4 mL from males. Blood was kept cool until centrifuged the same day and the plasma was transferred into solvent-rinsed glass culture tubes with glass pasteur pipettes and frozen at -20 ^{o}C. Fat biopsies were obtained from the same, blood-sampled terrapins. Fat biopsies of 0.2-1.2 g were taken through an incision in the lower left inguinal area and frozen in hexane-rinsed aluminum foil at -20 ^{o}C. Lidocaine was used to anesthetize the area and the incision was stitched with absorbable stitches. Terrapins were monitored for at least 48 hours before being released back to their capture site.

Tissue samples were also collected from a female terrapin that drowned during the field season. The female was initially frozen and later necropsied. Fat from the lower left inguinal area, liver, ovary, three follicles and 11 shelled eggs in the oviducts were collected with hexane-rinsed stainless steel instruments and frozen in hexane-rinsed aluminum foil. Whole blood was collected with a plastic Vacutainer tube (Becton, Dickinson and Company, Franklin Lakes, NJ) and frozen. Nineteen blue mussels (*Mytilus edulis*), a prey item of terrapins, were also collected from Forsythe in September 2006. They were frozen upon collection and later thawed, shucked, and tissues were homogenized as a single pool using a handheld stainless steel homogenizer.

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 and three isomers (alpha, beta and gamma) of HBCD. These were used to prepare seven calibration solutions at differing concentrations that were extracted and cleaned up alongside samples. Internal standard solutions were added to samples prior to extraction and 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, ^{13}C-labeled α-HBCD and γ-HBCD, 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.

Sample analysis

Plasma/blood samples (≈ 2 g) were equilibrated with an ethanol internal standard solution in a glass vessel for 12-18 hours. Formic acid, dichloromethane (DCM) to hexane (1:4 v:v) and a stir bar were added, and samples were extracted using a CEM Discovery Focused Microwave (CEM Corp., Matthews, NC) and cleaned up as described in Keller et al. (2009). A single pass of plasma/blood extracts through an acidified silica column followed by an alumina column using an automated solid phase extraction system (Rapid Trace SPE workstation, Caliper Life Sciences, Hopkinton, MA) was not sufficient, so extracts were cleaned up again using both columns. Fat, egg, tissue and mussel samples were minced and mixed with anhydrous Na_2SO_4, transferred to 33 mL pressurized fluid extractor cells, spiked with an *iso*-octane internal standard solution, and extracted

with DCM using pressurized fluid extraction (Dionex Corporation, Sunnyvale, CA) according to Keller et al. (2004a). These extracts were cleaned up using size exclusion chromatography with two Phenogel columns connected in-line with each other (a 600 mm x 21.5 mm and a 300 mm x 21.5 mm, both 10 µm particle size with 100 Å diameter pores; Phenomenex, Torrance, CA) and also with alumina columns. Egg and follicle samples required further clean-up using acidified silica columns.

Lipid content was determined gravimetrically from initial extracts prior to any clean-up. Approximately 5% to 10% by weight of each extract was removed and transferred to a tarred aluminum weighing pan. The solvent was allowed to evaporate at room temperature for 4-12 h, and the dried lipid residue was reweighed to the nearest 0.00001 g for fat and 0.0001 mg for plasma/blood.

Final extract volumes were approximately 0.2 mL. The extracts were analyzed on a gas chromatogram mass spectrometer (GC/MS; Agilent Technologies 6890N/5973 inert, Palo Alto, CA) using two injections. The first injection (20 µL) used a programmable temperature vaporization (PTV) inlet with a 30 m capillary column (DB-5MS, Agilent) and electron impact ionization mode to measure PCBs, pesticides, and most PBDE congeners. The second injection (2 µL) used cool on-column injection onto a 10 m DB-5MS column and negative chemical ionization mode to measure all PBDE congeners. Inlet, oven, carrier gas, and MS parameters were similar to the 30 m injection described in Keller et al. (2009) and the 15 m injection described within Method 3 of Stapleton et al.

(2007). The limit of detection (LOD) was determined by using the maximum ng in the lowest detectable calibration standard divided by the sample mass or the mean ng calculated in the procedural blanks plus three times the standard deviation all divided by the sample mass.

Statistical Analysis and Calculations

All data were normally distributed, except 4,4'-DDE and oxychlordane in plasma, which were log-transformed. Before statistical hypothesis testing and descriptive statistics were performed, concentrations <LOD for ΣPBDEs, ΣPCBs and 4,4'-DDE were set to half LOD. The whole blood POP concentrations of the necropsied female were converted to plasma concentrations using previously reported linear regressions between these blood compartments for loggerhead sea turtles (Keller et al., 2004b; Carlson, 2006). The statistical program SAS version 8.1 (SAS Institute Inc., Cary, NC) was used to test correlations between fat and plasma concentrations, as well as ANOVAs and MANOVAs to examine site and sex differences with tissue (plasma and fat) as co-variables. Lipid normalized concentrations were used only for correlations. Pearson correlation coefficients were obtained for comparisons between fat and plasma concentrations of 4,4'DDE, ΣPCBs and ΣPBDEs. A Spearman rank correlation coefficient was obtained for oxychlordane because the data did not fit assumptions of normality. MANOVAs were used when the compound of interest was measured in both fat and plasma samples (oxychlordane, 4,4'-DDE, ΣPCBs and ΣPBDEs). ANOVAs

were used for contaminants that were only detected in fat (HCB, dieldrin, 4,4'-DDD, 4,4'DDT, mirex and methyl-triclosan).

Principal component analyses (PCA) were performed to examine differences in the overall POP contaminant congener/compound patterns in the terrapin fat samples using MatLab (The Mathworks Inc., Natick, MA). Only compounds present in 80% or more of the samples (61 compounds) were chosen for the PCA, and concentrations of individual compounds were converted to percent of the sum POP concentrations, scaled and centered according to Swarthout et al. (2010).

Biomagnification factors (BMFs) were calculated as the ratio between the average lipid normalized concentration of the compound in terrapin fat and the lipid normalized concentration in the mussel tissue. BMFs were calculated using only fat values from terrapins captured at the Forsythe site because the mussels were only obtained from the Forsythe site. BMFs were calculated for the most abundant PCB congeners in fat (PCBs 99, 118, 138, and 153+132), PBDEs 47 and 153, and ΣPCBs, ΣPBDEs and 4,4'-DDE.

Results and Discussion

Quality Control

Concentrations measured in all three control materials were <10% different from certified values or past mean values for the majority of the compounds. The average percent difference for all reported values was 0.43%, 0.013% and 4.20% for SRM 1589a, Cc pool and SRM 1946, respectively.

Precision was assessed by comparing the two SRM 1946 replicates, which differed from each other by <5% for the majority of compounds.

Contaminant Concentrations

All compounds reported in Table 1 were detected in fat, including PBDE 209 although only in one sample. ΣPCBs, followed by 4,4'-DDE, oxychlordane, dieldrin and ΣPBDEs, were the predominant contaminants, which is similar to other reptiles (Swarthout et al., 2010). The presence of methyl-triclosan (a metabolite of triclosan, which is commonly found in antimicrobial soaps) in 14 of the 16 fat samples was noteworthy (Table 1). It was found in no other terrapin tissues analyzed, and no other study has attempted to measure it in any other reptile species. However, fish from Swiss lakes receiving effluent from waste water treatment plants (WWTP) had detectable levels of methyl-triclosan, 4.12 – 146 ng/g wet mass (Balmer et al., 2004). Terrapin methyl-triclosan levels ranged from <LOD to 5.25 ng/g wet mass (Table 1), yet the capture sites have no direct influence from a WWTP. Instead, all public sewage waste water is treated in Ocean County, NJ, and all effluent is pumped offshore into the Atlantic Ocean (www.ocua.com). Thus, the source of this compound leading to terrapin exposure is currently unknown.

Fewer compounds were detected in plasma and at lower concentrations than fat biopsies (Table 2). Certain compounds were removed during the acidified silica clean-up step (mirex, dieldrin, aldrin, endrin, methyl-triclosan and endosulfans), so these could not be reported in plasma (or eggs). HCB, 4,4'-

DDD, 4,4'-DDT, and PBDEs 47, 99 and 209 were <LOD. Similar to fat, ΣPCBs, followed by 4,4'-DDE, oxychlordane and ΣPBDEs, were the predominant contaminants (Table 2).

Terrapin fat biopsy and plasma POP concentrations were similar, within the same magnitude, in comparison to most other published concentrations in other turtle and reptile species (Table 3). However, terrapin plasma concentrations were much lower than the ΣPCBs measured in snapping turtles from highly contaminated Great Lakes sites and were much lower than 4,4'-DDE concentrations measured in American alligators (*Alligator mississippiensis*) in Lake Apopka (Table 3). Adult female terrapin plasma ΣPCB concentrations were more than twice the mean concentration reported in loggerhead sea turtles (*Caretta caretta*) from North Carolina. These comparisons are important to assess the risk of the terrapin to these contaminants. The lower loggerhead concentrations were significantly correlated with health parameters, such as white blood cell (WBC) counts, blood urea nitrogen, aspartate aminotransferase activity and magnesium levels (Keller et al., 2004c). The higher snapping turtle concentrations at contaminated Great Lakes sites have been related to feminized morphology compared to reference sites (De Solla et al., 1998). The higher 4,4'-DDE concentrations in male alligators from Lake Apopka are thought to be linked to feminized reproductive morphology (Guillette et al., 1999). Because terrapins in Barnegat Bay have plasma concentrations higher or similar to loggerhead sea turtles, they may be experiencing certain health effects and future studies should

examine their blood cell counts and plasma chemistries. They do not appear to be at risk for reproductive effects associated with their PCB, 4,4'-DDE and oxychlordane exposure, since terrapin plasma concentrations are much lower than those that have affected reproduction in snapping turtles and American alligators (Table 3).

Sex and Site Comparisons

Generally, males had higher concentrations for the majority of the compounds measured in fat than females, regardless of site (Table 1). Conversely, females had higher concentrations for the majority of the compounds measured in plasma than males (Table 2). Fat concentrations of oxychlordane, 4,4'-DDE, ΣPCB and ΣPBDE in males were significantly higher than females, and females had significantly higher concentrations of these predominant compounds in their plasma compared to males (Table 4). The last finding was unexpected. Adult males of a species typically have greater concentrations of lipophilic biomagnifying organohalogen compounds than adult females, because females off-load a proportion of their contaminant burden during reproduction (Pagano et al., 1999; Kelly et al., 2008; Moss et al., 2009). In other turtle studies, males have higher mean plasma concentrations than females for oxychlordane, mirex, 4,4'-DDE, ΣPBDEs and ΣPCBs (Table 3; Kelly et al., 2008).

Hypotheses that may explain the higher POP concentrations in female plasma involve their reproductive cycle and/or sexual dimorphism. Terrapins were captured in September after the nesting season which concludes around mid-

July and just before they brumate in response to cooler water temperatures (Brennessel, 2006). Both sexes may have been feeding at this time, but females may require more food during this season because they are larger than males and they produce the next set of follicles before brumation (Lee, 2003). Because the females are larger than males, they eat larger and more prey (Brennessel, 2006). Larger prey may have accumulated more POPs due to longer life span and position in the food web, as seen for another biomagnifying contaminant, methylmercury (Blanvillain et al., 2007). For both reasons (eating more for follicle development and eating more and larger prey), the females are likely exposed to higher levels of biomagnifying contaminants in the short-term in September, which would become apparent first in the circulating blood. Additionally, lipid mobilization for yolking follicles could also raise the female plasma POP concentrations. A combination of all these sex differences may explain the elevated female POP plasma concentrations. In future studies, plasma concentrations from males and females should be collected and analyzed for POPs throughout the active season to determine whether the increased female plasma POP concentrations are in response to feeding in preparation for follicle development and/or if the elevation is due in general to the larger female size and prey selection.

As expected, the majority of the compounds measured in both fat biopsies and plasma samples were higher in Spizzle, the site closer to industrial contamination sources, compared to Forsythe (Tables 1 & 2). Statistically, 4,4'-

DDE, 4,4'-DDD and 4,4'-DDT, ΣPCBs and ΣPBDEs were significantly different between sites (Table 4) . Spizzle was expected to have higher concentrations due to the influence of more industrial inputs in the northern portion of the bay such as high population and industrial density. Since terrapins show site fidelity and small home ranges as well as prey on sessile and slow moving prey items (mussels, snails, small crabs) their contamination levels should indicate the contamination of the area in which they are captured (Burger, 1977; Roosenburg, 1996; Gibbons et al., 2001; Mitro, 2003; Brennessel, 2006). Spatial differences in POP concentrations in another predator, the bottlenose dolphin (*Tursiops truncatus*), has been observed in another East coast bay, Biscayne Bay, near Miami, Florida. Dolphins captured closer to Miami in the more urban portion of the bay had higher concentrations than those in the further southern section of the bay (Litz et al., 2007). Both this previous study and the current study show that POP concentration gradients in estuarine predators are influenced by human abundance.

The sex * site interaction term of the ANOVA was significant for mirex and 4,4'-DDD, while the sex * site interaction term of the MANOVA was significant for ΣPCBs and ΣPBDEs, suggesting complex differences due to the inclusion of both sites and both sexes in the analyses (Table 4). The interaction term for mirex and 4,4'-DDD shows that Spizzle males had higher fat mirex and 4,4'-DDD concentrations than females, and Forsythe females had higher fat mirex and 4,4'-DDD concentrations than males (Table 1). Those results explain the

interaction within the fat biopsies only. A close examination of the sex * site interaction term for ΣPCBs and ΣPBDEs shows that the interaction is mostly explained by female plasma concentrations being greater then male plasma concentrations. Both of these values also had significant differences for sex and site. The biological significance interpreted from these results overall is that males had higher concentrations than females when both tissues were taken into account and Spizzle had higher concentrations than Forsythe, again when both tissues were taken into account as well as each tissue separately (Tables 1 and 2).

Turtles in general have been reported to be great indicators of chemical contamination in the environment (Meyers-Schone, 1994). In this study, significant differences in contaminants were found between two sites, 10 km apart which suggests that terrapin POP concentrations reflect the local POP contamination. On-going studies will further test the use of the terrapin as an indicator species with abiotic samples such as sediment analyses in corresponding capture sites. Because of the significant differences between male and female POP concentrations, in regards to the concentration level of a given contaminant, the data suggest sampling both sexes gives a more accurate range of contamination of a particular site. Terrapins are a suitable species for monitoring POP concentrations in Barnegat Bay based on the data reported in this study.

Contamination Patterns

A principal component analysis (PCA) showed that POP contaminant patterns in fat differed between sexes, but not sites (Figure 1). The first four PCs

accounted for 80% of the variance (PC1 = 36%, PC2 = 23%, PC3 = 12%, and PC4 = 9%). Sexes clustered separately when PC1 vs. PC 2 were plotted (data not shown) and when PC1 vs. PC4 were plotted (Figure 1). PC1 had high loadings for higher chlorinated PCBs and PC4 had high loadings for lower chlorinated PCBs, indicating that the sex-specific difference in POP pattern is driven by PCBs and not PBDEs or other pesticides. Lower chlorinated PCBs were more abundant in females, and higher chlorinated PCBs were more abundant in males (Figure 2). Similar results were reported in two freshwater turtle species from Tennessee where females of both species had higher proportions of lower chlorinated congeners, PCBs 99 and 118, and males had higher proportions of PCB 153+132 (Moss et al., 2009). Moss et al. [35] suggested that there may be differences in biotransformation between sexes or that females may be offloading certain congeners to eggs.

No discernable trend in PCB chlorination was observed between tissues. There were, however, more individual PCB congeners above 1% of the ΣPCBs in plasma than fat (Figure 2) and more individual congeners in females than males (data not shown). These data suggest that females may accumulate a broader suite of congeners than males.

The six most predominant congeners in descending order of abundance in fat and plasma, respectively, were PCBs 153+132 (39%), 118, 138, 180+193, 99, 183, and PCBs 153+132 (45%), 138, 99, 180+193, 118, 183. The top six congeners accounted for 74% and 78% of the ΣPCBs respectively for fat and

plasma (Figure 2). These patterns in terrapin fat and plasma are similar to marine turtle species, such as loggerhead and Kemp's ridley sea turtles (*Lepidochelys kempii*) with two exceptions (Keller et al., 2004a). In both terrapin fat and plasma, PCB 183 is in the top six most predominant congeners, whereas in the two sea turtles species it is replaced by PCB 187. In addition, although PCB 153 is the most abundant congener in the blood of all three turtle species, terrapins have double the proportion in comparison. This trend of a higher proportion of PCB 153+132 was also reported in two fresh water turtle species (Moss et al., 2009).

The pattern of PBDEs in the terrapin (Figure 3) is atypical when compared to other wildlife samples (Hites, 2004). In both plasma and fat samples hexa-brominated congeners (PBDEs 153 and 154) and PBDE 100 were more abundant than PBDE 47 and 99 (Figure 3). This atypical pattern cannot be explained by problems with the analytical methodology, because the PBDE concentrations measured in the human serum and fish tissue SRMs, which have typical patterns, were accurate. Terrapin plasma had only four detectable congeners with PBDE 154 accounting for 43% of the total followed by PBDEs 153, 100 and 183. Surprisingly, PBDEs 47 and 99 were not detected in any plasma sample (Table 2). The respective LODs for PBDE 47 and 99 were 47.4 and 53.1 pg/g wet mass. Similarly, atypical patterns of hexa-PBDEs occurring at proportions larger than expected have been reported in a few other turtle species. Common musk turtles (*Sternotherus odoratus*) and Cumberland sliders (*Trachemys scripta troostii*)

sampled in Tennessee had plasma patterns with PBDE 100 as the predominant congener followed by PBDEs 153, 154, 47 and 99 (Moss et al., 2009). Loggerhead sea turtle plasma from North Carolina had PBDE 154 and 100 as the predominant congeners and PBDE 47 being the least predominant (Carlson, 2006). A similar observation was reported in loggerhead sea turtle eggs from North Carolina where PBDEs 154 was reported at a similar proportion as 47 followed by 100, 99 and 153 (Keller et al., 2005). Another interesting pattern in terrapin plasma is the presence of PBDE 183 in 8 of 16 turtles and was more abundant than PBDE 47 and 99 (Table 2). PBDE 183 does not typically predominate in biotic samples, but it has been observed in male Cumberland sliders and loggerhead sea turtle plasma, but at concentrations lower than PBDE 47 in most cases (Carlson, 2006; Moss et al., 2009).

Terrapin fat had similar PBDE patterns to terrapin plasma except there were more individual PBDE congeners detectable in the fat. Overall in fat, PBDE 153 was the most abundant, accounting for 30% of the total, followed by PBDEs 100, 154, 99, 183, 155 and 47. PBDE 183 was also the fourth most abundant congener and was detected in 14 out of 16 turtles (Table 1). There were not any discernable differences in PBDE bromination between terrapin tissues, sexes or sites. To our knowledge, this study is the first to report this atypical PBDE pattern in fat for turtles or any other reptile species, as well as being the first to report PBDE concentrations and profiles in a solely estuarine reptile. This atypical profile, while reported in a select few freshwater, marine and now a

strictly estuarine turtle species is not representative of all turtle species. The normal pattern observed in wildlife, with PBDE 47 and 99 being the predominant congeners, has been reported in the plasma of three marine turtle species from Australia, loggerhead samples from SC and FL, Kemp's ridley blood from the southeastern US and Gulf of Mexico and green turtle (*Chelonia mydas*) plasma from the Gulf of Mexico (Keller et al., 2005; Hermanussen et al., 2008; Swarthout et al., 2010). The reason for this atypical pattern is unknown but is assumed to be due to biotransformation or metabolism of certain PBDE congeners, and appears to be more related to capture location (northern latitudes in the U.S.) rather than species specific.

Tissue Comparisons

Statistically significant correlations were observed between contaminant concentration in fat biopsies and those in plasma samples for oxychlordane, ΣPCBs and ΣPBDEs (Figure 4). ΣPBDEs had the strongest significant correlation $r=0.945$, $p>0.0001$ followed by ΣPCBs with $r=0.935$, $p>0.0001$. Oxychlordane had a weak correlation, $r=0.521$ that was marginally significant, $p=0.046$. There was no significant correlation for 4,4'-DDE. These results are similar to those reported in loggerhead sea turtles, where significant correlations were observed for ΣPCBs, ΣDDTs (which was mainly 4,4'-DDE) and Σchlordanes (which included oxychlordane) (Keller et al., 2004a). It was surprising that terrapin tissue correlations were not significant for 4,4'-DDE based on the strong and significant correlation seen in loggerhead tissues. Due to the significant

correlations in terrapins for oxychlordane, ΣPCBs and ΣPBDEs, plasma can be viewed as a preferred matrix for monitoring organic contaminants in reptiles. Strong correlations between terrapin tissues suggest that plasma concentrations can predict the concentration of most POPs in the more long-term storage depots like fat. Blood collection is non-lethal and much less invasive than surgically removing fat biopsies. Blood sampling from live turtles also minimizes biases associated with opportunistic sampling of deceased specimens.

POP concentrations measured in various tissues of the necropsied female are reported in Table 5. For all compounds reported, the concentrations on a wet mass basis were greatest in the fat followed by the follicles, eggs, liver then whole blood. This is comparable to the tissue distribution of PCBs reported in snapping turtles and loggerhead sea turtles. The distribution from greatest to least concentration of PCBs of two male snapping turtles was fat > testes > brain > liver > kidney > pancreas > lungs and the distribution in male and female loggerheads was liver > kidney > heart > lung > muscle (Bryan et al., 1987; Storelli and Marcotrigiano, 2000). In the terrapin tissues, POP concentrations followed the order of lipid content as expected, suggesting that POP partitioning is dependent on the amount of lipid (Table 5). The gravid female whole blood and fat concentrations were similar in magnitude to other terrapins sampled. There were no obvious differences in patterns of PCBs and pesticides between the gravid female tissues or compared to the other terrapins sampled. All tissues sampled had the atypical PBDE profile discussed in the previous section, except

PBDE 155 was more abundant in this female (fourth most predominant congener) than the other terrapin fat samples (Table 5; Figure 3).

The entire clutch of terrapin eggs collected from the gravid female as well as the three follicles had detectable levels of compounds observed in other tissues of the gravid female (Table 5). POP concentrations for eggs and follicles were less than those in the fat but greater than those reported in the whole blood as was expected due to the respective lipid content of each tissue (Table 5). The egg and follicle PCB and PBDE patterns were also very similar to the other tissues, especially compared to the fat. The top 5 predominant PCB congeners were found in the same order in the fat, eggs and follicles and accounted for 70%, 73% and 70% of the ΣPCBs in each tissue, respectively. The atypical PBDE pattern was also observed in the eggs and follicles, similar to other tissues of this female.

It is important to note the female died with the entire clutch inside the oviducts so the eggs had not been exposed to the outside environment. Thus this study provides data showing maternal transfer of not only PCBs, which have been suspected to maternally transfer in other turtle species (Pagano et al., 1999; Kelly et al., 2008), but also PBDEs and the other pesticides reported in Table 5. Since the entire clutch was collected and the location of each egg within each oviduct was noted, intra-clutch variability for POP concentrations could be determined. The concentrations were very similar among all 11 eggs. The small standard deviations and narrow ranges (Table 5) suggest that contaminants are evenly distributed to the follicles during egg development and concentrations do not

depend upon the order of egg laying within a clutch. The relative standard deviations (RSDs) were <15% with the majority being <10%. Relatively even distribution of POPs within a single clutch has been reported in loggerhead sea turtles (Alava et al., 2006), however a study has shown that POP concentrations can depend on order of egg laying in snapping turtles (Bishop et al., 1995). The current study suggests that any single egg from a terrapin clutch would be representative of the entire clutch and the mother's contaminant burden. Since we sampled the clutch from only one individual female more data are needed to establish that intra-clutch variation is characteristically low for the diamondback terrapin.

In a pool of 19 mussels (*Mytilus edulis*) collected in the less impacted Forsythe site all pesticides except the DDTs were <LOD (Table 5). PCBs were the most abundant compound class with PCB 153+132 being the most abundant congeners accounting for 22% of the ΣPCBs, followed by PCBs 187, 118, 149, 101 and 99. The top six congeners only accounted for 57% of the PCBs. This pattern differs from the terrapin tissues in that 18 more congeners contributed more than 1% of the ΣPCBs in mussels than terrapins. Most of these additional congeners were lower chlorinated, possibly suggesting terrapins are capable of eliminating or biotransforming these PCBs or that the PCB pattern may have been influenced by the digestive glands of the mussels. PCB metabolism has been reported for fish, bird and reptile species (Schlezinger et al., 2000). Only three PBDE congeners were detected in the mussels, PBDE 47, 153 and 209 (Table 5).

PBDE 209 accounted for 45% of the total followed by PBDE 47 (37%) and PBDE 153 (18%). This congener pattern is similar to other mussel studies and wildlife patterns in which PBDE 47 is in greater proportion than hexa-PBDEs (Hites, 2004; Wang et al., 2009). The presence of PBDE 209 at such a high proportion compared to other wildlife species can be easily explained by the suspension feeding behavior of mussels and that this study used a homogenate of the entire mussel without the shell. PBDE 209 is known to associate with sediments at very high concentrations so the ingestion of sediments by mussels and inclusion of this material within the mussel digestive tract would result in a large concentration of PBDE 209 (van Leeuwen, 2008).

Biomagnification Factors

Biomagnification factors (BMFs) were calculated for compounds with concentrations reported in both mussel and fat tissue on a ng/g lipid basis (Table 6). All BMFs reported are greater than one, as expected based on their lipophilic nature (Table 6). One notable exception is PBDE 47, which had a BMF of only 0.33. Biomagnification of PCBs and PBDEs has been reported in a food web in Lake Michigan, and 4,4'-DDE in a food web in a high mountain lake in Spain (Stapleton and Baker, 2003; Catalan et al., 2004). BMFs and log K_{ow} have been reported to correlate up to a log K_{ow} of 7.36 for compounds in general, and up to log K_{ow} of 8.18 for PCB congeners (Burreau et al., 2004). All compounds reported in Table 6 have log K_{ow} from 5.5 to 7.9; therefore, they were all expected to biomagnify from mussels to terrapin tissue (Table 6). The low BMF for PBDE

47 is surprising, especially since this congener has been reported to have very high uptake efficiencies in rats (82%) and fish (>90%) (Burreau et al., 1997; Staskal et al., 2005). BMFs of PBDE 47 reported in other species such as salmon (BMF = 3.5-11) and gray seals (*Halichoerus grypus*) (BMF = 19) are much greater than one (De Wit, 2002). A BMF <1 for PBDE 47 in terrapins suggests elimination or biotransformation. Distribution of PBDE 47 to another tissue was ruled out by analyzing the other tissues of the gravid female (Table 5), all of which had a similar PBDE pattern. Debromination of PBDE 183 to PBDE 154 and PBDE 99 to 47 has been reported to occur in the intestinal tract of the common carp (*Cyprinus carpio*) (Stapleton et al., 2004a). Hydroxylated PBDEs (OH-PBDEs) have been reported in rats and fish, suggesting an additional mechanism of biotransformation of specific congeners (Malmberg et al., 2004; Valters et al., 2005). Future studies should assess the uptake of PBDE 47 into the terrapin and whether it is capable of debrominating or hydroxylating this congener. This is important because formation of OH-PBDEs may result in effects on thyroid hormone homeostasis (Marsh et al., 1998; Darnerud, 2008). An on-going study is attempting to correlate plasma thyroid hormone levels and PBDE concentrations in terrapins.

Even though PBDE 99 was not detected in the mussel sample, it was still found in lower than normal proportions in terrapins as compared to other wildlife patterns. It is possible that PBDE 99 may be going through a similar process as PBDE 47 in terrapins since it is expected to be found in other terrapin prey items.

This study confirmed that terrapins are good indicators of organic contamination in the estuarine ecosystem, as has been suggested previously (Kannan et al., 1998; Blanvillain et al., 2007). This study also provides data strongly suggestive of maternal transfer of POPs as well as useful methods for sampling terrapins in the most non-invasive or non-destructive ways. Collecting plasma is non-lethal, relatively easy to collect and represents the stored contaminant burden for the organism, at least for most POPs. Additionally, this study showed that a single egg from a nest may represent the mean POP contamination of the clutch as well as the adult female, which can reduce the impact of sampling on terrapin populations. The terrapins of Barnegat Bay have POP levels lower than certain impacted reptile populations, but higher than others that show significant correlations with health endpoints. Terrapins showed an atypical accumulation pattern for PBDEs compared to other wildlife. Future terrapin studies should focus on the reproductive and health effects of these contaminants at environmentally-relevant levels. Effects observed in terrapins can indicate possible threats to the estuarine ecosystem because of the biotic and abiotic relationships the model species shares with different habitats and species of the Bay.

Table 1. Summary of POP concentrations (ng/g wet mass) in fat biopsies from diamondback terrapins from Barnegat Bay, New Jersey. Only predominant PCB and PBDE congeners are listed.

	Forsythe Female n=6		Forsythe Male n=5		Spizzle Female n=3		Spizzle Male n=3		All Terrapins n=17			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean (SD)	Median	Range	n>LOD
HCB	0.652	0.424	0.916	0.521	1.16	1.19	1.17	0.584	0.909 (0.640)	0.678	0.178-2.51	16
Oxychlordane	70.7	32.9	65.1	6.63	51.3	20.7	112	48.4	73.4 (34.1)	68.4	27.4-148	16
Mirex	4.821	2.86	4.35	2.87	4.36	2.12	11.1	3.92	5.79 (3.73)	4.52	1.46-14.3	16
Me-triclosan	1.55	1.86	5.25*	NA	0.323*	NA	<LOD	NA	2.04 (2.29)	0.831	<LOD-5.25	14
Dieldrin	27.6	11.7	38.8	16.0	27.4	4.91	29.5	12.1	29.6 (10.7)	25.8	<LOD-50.1	5
4,4'-DDE	184	56.1	166	59.3	233	32.2	288	107	208 (74.4)	199	27.4-148	16
4,4'-DDD	2.33	1.47	2.33	NA	2.62	1.23	3.65	0.774	2.70 (1.25)	2.33	<LOD-5.10	13
4,4'-DDT	1.75	0.627	1.73	1.33	2.77	0.766	2.62	0.785	2.13 (0.895)	2.06	<LOD-3.65	15
PCB 99	82.8	42.7	61.6	17.9	110	24.6	160	38.7	100 (47.2)	91.9	31.7-198	15
PCB 118	133	69.4	135	81.9	157	25.2	4.84	102	177 (106)	165	32.8-411	16
PCB 153+132	374	191	15.5	244	407	104	1477	467	636 (485)	487	116-1780	16
PCB 138	106	54.4	87.1	42.0	122	20.4	246	52.3	131 (72.0)	131	28.0-294	16
PCB 180+193	57.6	27.9	111	39.9	56.9	12.5	250	84.5	107 (84.4)	75.6	19.7-318	16
ΣPCBs	1050	506	1330	780	1210	202	3380	998	1585 (1050)	1340	323-4070	16
PBDE 47	1.07	0.633	1.04	0.336	1.21	0.414	1.31	0.145	1.13 (0.457)	1.18	0.300-1.86	14
PBDE 99	1.60	1.63	1.05	1.47	1.28	1.12	1.23	0.914	1.33 (0.491)	1.19	0.874-2.83	16
PBDE 100	3.44	0.695	2.05	0.263	3.74	0.298	3.66	0.150	3.19 (1.44)	3.41	0.773-5.11	16
PBDE 154	3.35	2.08	1.43	1.57	3.67	0.804	3.66	1.96	2.99 (1.85)	3.06	0.340-5.84	16
PBDE 153	3.60	2.35	4.72	3.50	1.28	1.61	21.8	7.54	7.30 (8.01)	4.47	0.560-27.3	16
PBDE 183	0.638	0.239	1.38	0.649	1.22	1.03	8.97	2.42	2.76 (3.55)	0.973	0.467-11.6	14
PBDE 209	0.349*	NA	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.349*	NA	NA	1
ΣPBDEs	15.9	7.87	13.0	9.24	16.7	4.26	43.6	12.0	20.5 (13.9)	17.8	4.80-50.7	16
% Lipid	67.2	11.3	60.7	1.42	71.8	5.15	58.9	7.34	64.9 (8.77)	64.6	48.1-81.0	16

* measurement is only one sample, therefore no standard deviation
<LOD = below limit of detection
SD = standard deviation

Table 2. Summary of POP concentrations (ng/g wet mass) in plasma from diamondback terrapins from Barnegat Bay, New Jersey. Only predominant PCB and PBDE congeners are listed.

	Forsythe Female n=5		Forsythe Male n=5		Spizzle Female n=3		Spizzle Male n=3		All Terrapins n=16			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean (SD)	Median	Range	n>LOD
HCB	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
oxychlordane	0.450	0.269	<LOD	<LOD	0.357	0.142	<LOD	<LOD	0.419 (0.223)	0.387	<LOD-0.827	6
4,4'-DDE	1.55	1.68	0.240	NA	3.43	2.10	1.26	1.84	1.84 (1.87)	1.04	<LOD-5.66	12
PCB 99	1.36	0.857	0.175	0.07	1.89	1.09	0.566	0.281	0.990 (0.916)	0.697	<LOD-2.82	15
PCB 118	1.01	0.835	0.235	0.08	2.21	0.466	0.477	0.301	0.847 (0.831)	0.399	<LOD-2.54	14
PCB 153+132	4.37	2.71	2.08	1.66	6.83	4.26	4.62	2.95	4.16 (3.03)	3.12	0.615-9.56	16
PCB 138	1.33	0.910	0.217	0.16	2.25	1.25	0.861	0.390	1.07 (1.00)	0.767	0.031-3.11	16
PCB 180+193	0.638	0.355	0.361	0.32	0.901	0.521	0.756	0.452	0.623 (0.408)	0.519	0.078-1.26	16
ΣPCBs	12.4	8.13	3.82	2.94	18.5	10.7	9.58	5.21	10.3 (8.24)	8.19	1.02-26.3	16
PBDE 47	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
PBDE 99	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
PBDE 100	0.086	0.037	<LOD	<LOD	0.129	0.016	0.100	NA	0.100 (0.034)	0.108	<LOD-0.140	7
PBDE 154	0.072	0.047	0.034	0.01	0.113	0.060	0.065	0.040	0.071 (0.047)	0.046	<LOD-0.149	14
PBDE 153	0.039	0.021	0.031	0.02	0.056	0.038	0.061	0.039	0.047 (0.029)	0.048	<LOD-0.105	12
PBDE 183	0.010	<LOD	0.088	<LOD	0.015	0.003	0.053	0.008	0.038 (0.028)	0.031	<LOD-0.088	8
PBDE 209	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
ΣPBDEs	0.174	0.124	0.050	0.075	0.269	0.172	0.212	0.142	0.160 (0.138)	0.131	<LOD-0.377	14
% Lipid	1.18	0.383	0.297	0.057	1.70	0.699	0.339	0.158	0.855 (0.657)	0.815	0.240-2.35	16

<LOD = below limit of detection
SD = standard deviation

Table 3. Comparison of means of persistent organic pollutants in ng/g wet mass, unless otherwise indicated, reported in blood and fat samples of reptiles.

Species	Tissue	Sex Stage	Year	Location	Oxychlordane	4,4'-DDE	Dieldrin	Mirex	Sum PCBs	Sum PBDEs	n	Reference
N.Diamondback terrapin	plasma	AM	2006	Barnegat Bay, NJ	<LOD	1.00	NR	NR	5.98	0.111	8	Current Study
N.Diamondback terrapin	plasma	AF	2006	Barnegat Bay, NJ	0.419	2.25	NR	NR	14.7	0.209	8	Current Study
Common musk	plasma	AM	2004	Tennessee	0.322	1.28	0.193	0.101	11.5	1.24	5	Moss et al., 2009
Common musk	plasma	AF	2004	Tennessee	0.215	0.738 [d]	0.257	0.081	11	0.809	5	Moss et al., 2009
Cumberland slider	plasma	AM	2004	Tennessee	0.131	0.538	0.163	0.107	14.3	0.885	5	Moss et al., 2009
Cumberland slider	plasma	AF	2004	Tennessee	0.057	0.576	0.162	0.038	6.57	0.46	5	Moss et al., 2009
Snapping turtle	plasma	AM	1995	Cootes Paradise, Lake Ontario [a]	7	10.1	0	8.3	414.8	NR	9	De Solla et al,. 1998
Snapping turtle	plasma	AM	1995	Lynde Creek, Lake Ontario [a]	2.4	21.7	3.8	10	263.3	NR	2	De Solla et al,. 1998
American Alligator	serum	JM	1995	Lake Apopka, FL [a,b]	0.39	7.35	1.68	0.28	1.2 [c]	NR	6	Guillette et al,. 1999
Loggerhead sea turtle	plasma	JM & JF	1998-2006	North Carolina	0.0308	0.24	NR	NR	3.78	0.066	45	Carlson 2006
Loggerhead sea turtle	plasma	JM & JF	2003	Southeast US	NR	NR	NR	NR	2.53	0.131	29	Keller et al., 2005
Loggerhead sea turtle	whole blood	JM & JF	2000-2001	North Carolina [a]	0.225 [f]	0.649 [e]	0.061	0.045	5.56	NR	48	Keller et al., 2004b
Kemp's Ridley sea turltle	whole blood	JM & JF	1999	North Carolina [g]	0.10	0.765	0.083	0.033	4.54	NR	8	Keller et al., 2004a
Kemp's Ridley sea turltle	whole blood	JM & JF	2001	Southeast US	1.22 [f]	1.49 [e]	0.608	0.0092	10.7	0.148	3	Swarthout et al., 2010
Kemp's Ridley sea turltle	whole blood	JM & JF	2001-2002	Gulf of Mexico	0.113 [f]	0.686 [e]	0.225	0.0152	4.27	0.23	46	Swarthout et al., 2010
Green sea turtle	whole blood	JM & JF	2001-2002	Gulf of Mexico	0.0112 [f]	0.128 [e]	NR	0.0147	0.534	0.158	9	Swarthout et al., 2010
Green sea turtle	whole blood	All Included	2004-2008	Queensland Australia	NR	NR	NR	NR	NR	0.00444	7 pooled	Hermanussen et al., 2008
Flatback sea turtle	whole blood	AF	2004-2008	Queensland Australia	NR	NR	NR	NR	NR	0.00609	1	Hermanussen et al., 2008
Hawksbill sea turtle	whole blood	JF	2004-2008	Queensland Australia	NR	NR	NR	NR	NR	0.013	1	Hermanussen et al., 2008
N.Diamondback terrapin	fat	AM	2006	Barnegat Bay, NJ	85.3	218	33.2	7.23	2210	26.1	7	Current Study
N.Diamondback terrapin	fat	AF	2006	Barnegat Bay, NJ	64.2	200	27.5	4.67	1100	16.2	9	Current Study
Loggerhead sea turtle	fat	JM & JF	2000-2001	North Carolina [a]	35.2 [g]	64.4	9.28 [g]	11.5 [g]	256	NR	44	Keller et al., 2004c
Kemp's Ridley sea turltle	fat	JM & JF	1998-2000	North Carolina	72.5 [g]	90.9	32.1 [g]	3.31 [g]	525	NR	10	Keller et al., 2004a
Snapping turtle	fat	AF		Superfund Sites, NY [g]	NR	36.8	NR	1940	180,000	NR	3	Pagano et al., 1999
Snapping turtle	fat	AF		Non-Superfund Sites, NY [g]	NR	301	NR	13.4	913	NR	2	Pagano et al., 1999

Abbreviations: A = adult, F = female, J = juvenile, <LOD = below limit of detection, M = male, NR = not reported.

[a] Sites where health effects were associated with reptile contaminant concentrations, including altered endocrine parameters, reproductive and developmental effects as well as correlations with blood cell counts or plasma chemistries.
[b] mean in ng mL^{-1}.
[c] estimated values from a graph.
[d] Value excludes one turtle with unusually high concentrations of p,p'-DDE (29.9 ng g^{-1}).
[e] Total DDTs were reported.
[f] Total chlordanes were reported.
[g] Mean values converted from lipid normalized values using mean lipid content.

Table 4. Summary of statistical tests performed and p-value results. Two sites compared, Forsythe (south) and Spizzle (north) and both sexes. Results in bold are significant, ($p<0.05$).

	Statistical Test Used*	Site	Sex	Sex by Site Interaction
HCB	ANOVA	0.2116	0.8634	0.8866
Oxychlordane	MANOVA	0.3100	**0.0015**	0.0807
Mirex	ANOVA	0.0651	0.0655	**0.0380**
Me-triclosan	ANOVA	0.3770	0.6726	0.5877
Dieldrin	ANOVA	0.9776	0.7890	0.9598
4,4'-DDE	MANOVA	**0.0259**	**0.0007**	0.4169
4,4'-DDD	ANOVA	**0.0106**	0.1913	**0.0276**
4,4'-DDT	ANOVA	**0.0303**	0.5792	0.8188
ΣPCBs	MANOVA	**0.0190**	**0.0004**	**0.0271**
ΣPBDEs	MANOVA	**0.0199**	**0.0032**	**0.0172**

*MANOVAs included data from both plasma and fat biopsies. ANOVAs only include data from fat biopsies.

Table 5. Persistent organic pollutants (ng/g wet mass) in tissue samples from a necropsied gravid female diamondback terrapin and a composite of blue mussels (*Mytilus edulis*).

	Whole Blood	Fat	Liver	Ovary	Follicles (n=3)		Eggs (n=11)			Mussels
	n=1	n=1	n=1	n=1	Mean	SD	Mean	SD	Range	n=1, pool of 19
HCB	<LOD	0.178	0.059	<LOD	<LOD	<LOD	0.044	0.007	<LOD-0.055	<LOD
Oxychlordane	<LOD	51.6	2.38	1.19	11.4	2.11	6.95	0.641	6.08-8.47	<LOD
Mirex	NR	8.33	0.282	0.147	0.970	0.040	0.455	0.066	0.370-0.551	<LOD
Me-triclosan	NR	<LOD	<LOD	<LOD	NR	NR	NR	NR	NR	<LOD
Dieldrin	NR	16.8	<LOD	<LOD	NR	NR	NR	NR	NR	<LOD
4,4'-DDE	0.485	131	5.18	2.80	25.3	0.813	16.3	1.65	14.0-20.0	1.35
4,4'-DDD	NR	1.20	0.122	<LOD	0.349	0.095	0.075	0.011	0.061-0.099	0.568
4,4'-DDT	NR	0.821	<LOD	<LOD	0.117	0.033	0.076	0.009	0.063-0.092	0.045
PCB 99	0.491	99.4	3.85	2.07	22.9	0.963	14.3	1.31	12.8-17.1	0.186
PCB 118	0.625	195	8.22	4.41	43.3	5.80	25.9	2.47	22.9-31.1	0.231
PCB 153+132	2.72	597	23.5	12.3	104	12.3	58.9	5.85	52.4-69.7	0.739
PCB 138	0.887	164	6.62	3.59	29.0	4.44	16.8	1.71	14.8-20.1	0.084
PCB 180+193	0.409	96.8	3.43	1.89	13.0	1.17	6.86	0.717	6.02-8.07	0.047
ΣPCBs	7.11	1672	67.3	34.3	291	25.4	171	16.9	152-203	3.27
PBDE 47	<LOD	0.610	<LOD	<LOD	0.075	0.006	0.049	0.013	0.031-0.076	0.048
PBDE 99	<LOD	1.11	<LOD	<LOD	0.212	0.115	0.090	0.011	0.398-0.514	<LOD
PBDE 100	<LOD	5.11	0.179	0.100	0.751	0.101	0.445	0.037	0.071-0.106	<LOD
PBDE 154	0.039	5.32	0.186	0.112	0.764	0.040	0.346	0.029	0.314-0.399	<LOD
PBDE 153	0.023	6.87	0.235	0.129	0.893	0.078	0.414	0.046	0.357-0.511	0.022
PBDE 183	<LOD	0.989	<LOD	<LOD	0.234	0.067	0.089	0.010	0.076-0.107	<LOD
PBDE 209	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.058	NA	NA	0.058
ΣPBDEs	0.062	24.0	0.683	0.418	3.69	0.801	1.69	0.144	1.48-1.94	0.128
% Lipid	0.494	48.1	3.19	1.59	15.1	0.648	8.13	1.21	5.95-10.77	0.979

<LOD = below the limit of detection
SD = standard deviation

Table 6. Biomagnification factors (BMF) for POPs in diamondback terrapin fat tissue and blue mussel (*M. edulis*) prey from Forsythe, Barnegat Bay, NJ.

Compound	Mean Fat Conc. ng/g lipid	Mussel Conc. ng/g lipid	BMF	log K_{ow}
HCB	1.12	<LOD	-	5.5
oxychlordane	107	<LOD	-	6.1-6.4
Mirex	7.41	<LOD	-	6.89
Dieldrin	46.8	<LOD	-	5.48
4',4-DDE	274	138	1.98	5.69
PCB 99	118	19.0	6.20	6.39
PCB 118	212	23.6	9.0	6.74
PCB 153+132	726	75.5	9.6	6.9, 6.6
PCB 138	157	8.59	18.3	6.83
PCB 180+193	128	4.80	26.7	7.4-7.5
ΣPCBs	1860	334	5.56	NA
PBDE 47	1.60	4.87	0.33	6.81
PBDE 153	6.5	2.29	2.86	7.9
ΣPBDEs	23.3	13.1	1.78	NA

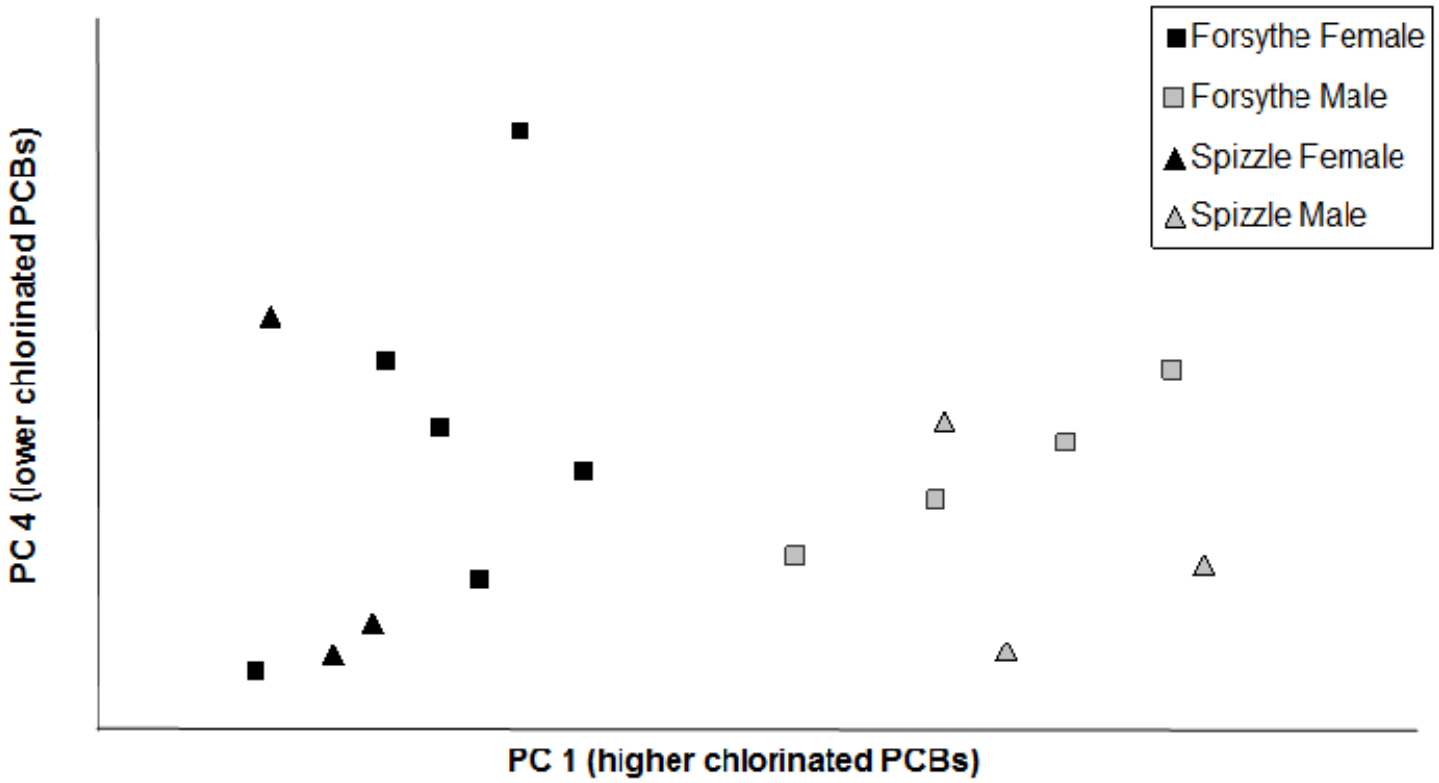

Figure 1. Principal component scores of POP patterns in fat biopsies from diamondback terrapins from Barnegat Bay, New Jersey. Loadings of principal component 1 (PC1) are large for PBDEs 47, 100, 155, 154 and higher chlorinated PCBs. PC4 loadings are large for lower chlorinated PCBs.

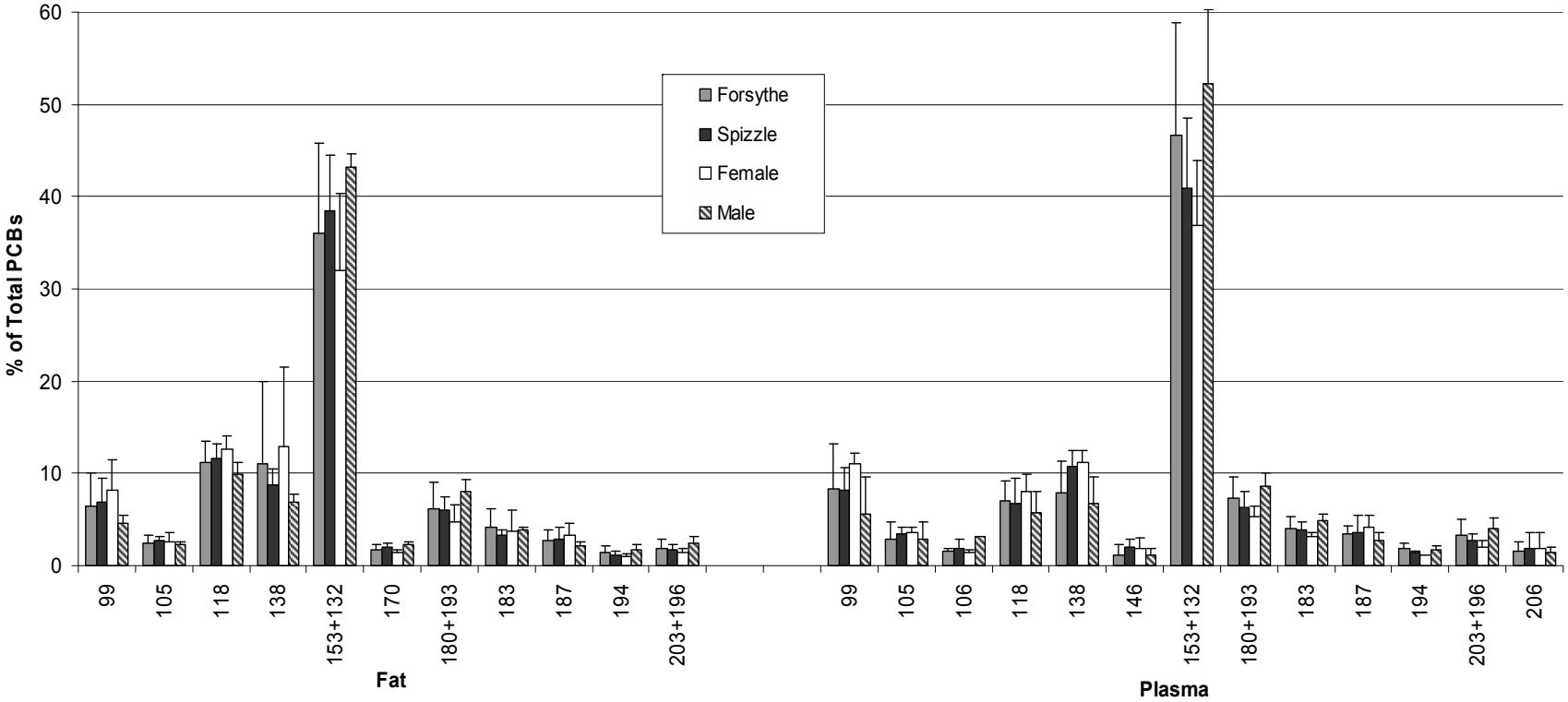

Figure 2. Diamondback terrapin PCB congener patterns in fat and plasma. Congeners listed are those above 1% of total PCBs and are present in all four categories (Forsythe, Spizzle, Female, Male). Error bars represent one standard deviation.

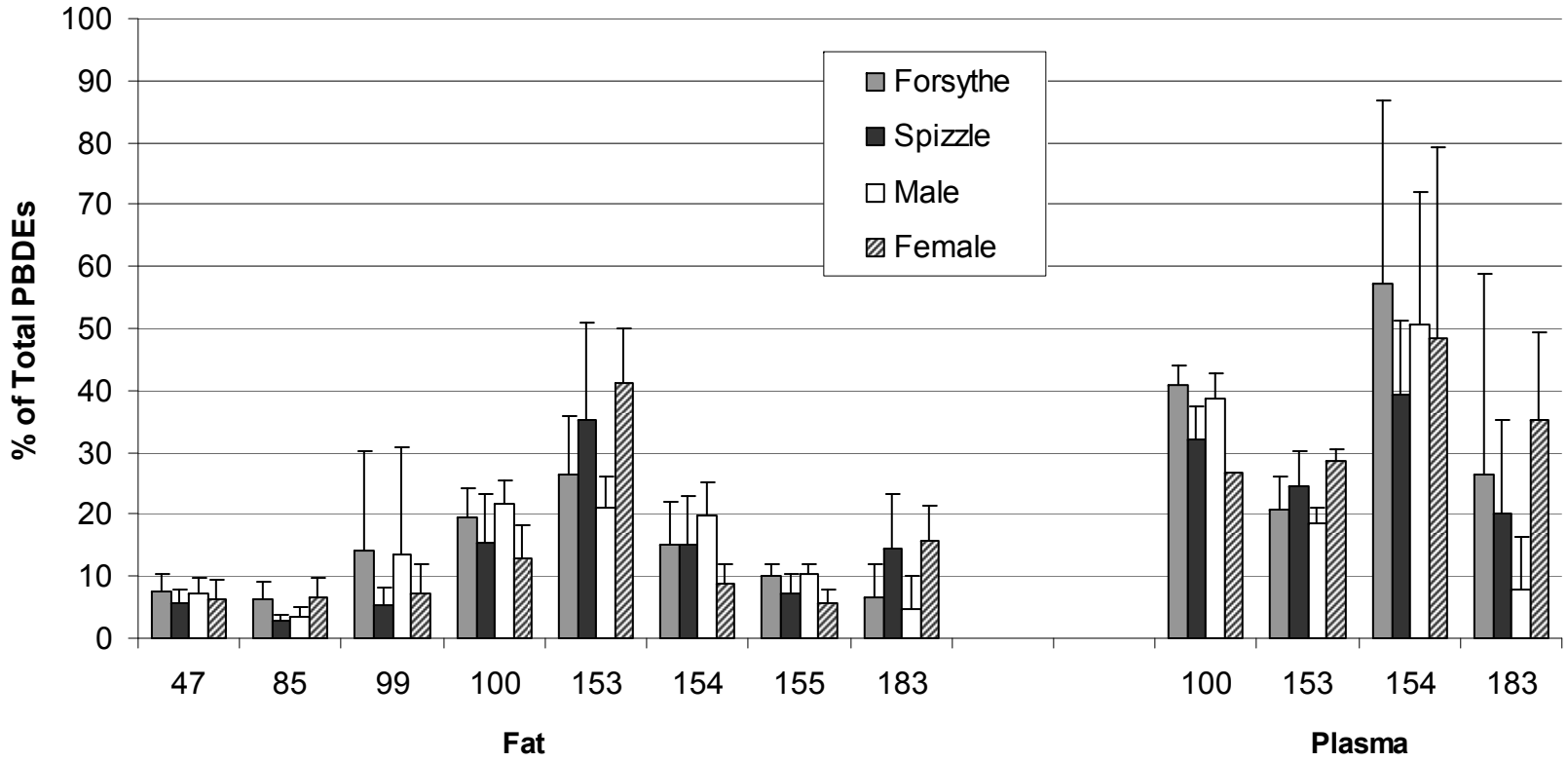

Figure 3. Diamondback terrapin PBDE congener patterns in fat and plasma. Congeners listed are those above 1% of total PBDEs and are present in all four categories (Forsythe, Spizzle, Female, Male). Error bars represent one standard deviation.

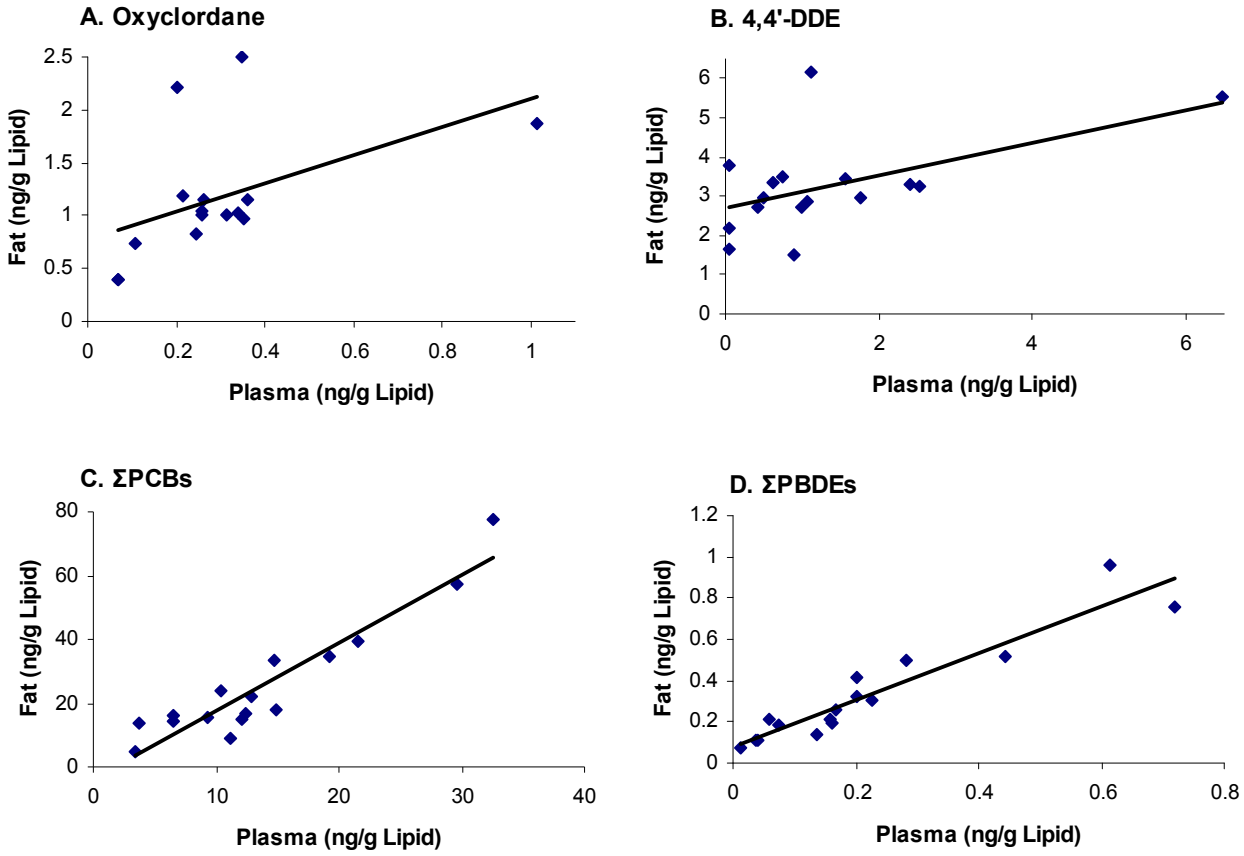

Figure 4. Figure 4. Relationships between fat biopsy and plasma concentrations of POPs in diamondback terrapins. Concentrations are lipid normalized. Linear regression statistics are given below, followed by either Pearson (4,4'-DDE, ΣPCBs and ΣPBDEs) or Spearman rank correlation (oxychlordane) statistics. For Oxychlordane and 4,4'-DDE equations the statistics were run using log-transformed values, however the figure depicts the non-transformed values to show the concentration values.

(A) Oxychlordane $y = 0.573 x + 0.371$, $r^2 = 0.563$, $p=0.0013$, $r_s = 0.52143$, $p=0.0462$.

(B) 4,4'-DDE $y = 0.116 x + 0.504$, $r^2=0.2126$, $p=0.0722$, $r_p=0.4611$, $p=0.0722$.

(C) ΣPCBs $y = 2.29 x - 3.70$, $r^2=0.8743$, $p<0.0001$, $r_p=0.93504$, $p<0.0001$.

(D) ΣPBDEs $y = 1.14 x + 0.078$, $r^2=0.8926$, $p<0.0001$, $r_p=0.94479$, $p<0.0001$.

CHAPTER 3:
PERSISTENT ORGANIC POLLUTANTS IN GRAVID DIAMONDBACK TERRAPIN PLASMA, EGGS AND NESTING SEDIMENTS IN BARNEGAT BAY, NJ

Abstract

Persistent organic pollutants (POPs) were measured in diamondback terrapin tissues and nesting beach sediments to further characterize the occurrence and patterns of POP contamination in Barnegat Bay, New Jersey. Samples were extracted and analyzed using gas chromatography mass spectrometry. Maternal transfer of PCBs, PBDEs, and OCPs in the diamondback terrapin was validated by the presence of these contaminants in terrapin eggs that were collected directly after ovoposition. Contaminant concentrations in plasma, collected from each of the 15 females, were not significantly correlated with egg concentrations. The unexpected lack of correlation may be due to the fact that the majority of follicular development occurs in late summer and fall, and the contaminants from maternal diet at that time are the contaminants incorporated into the developing embryo. Therefore the plasma samples may not reflect the POPs associated with the diet of the terrapin at the time of follicular development. Significant differences were seen between two sampling sites within Barnegat Bay, providing validation that terrapins are suitable indicators of local POP contamination. Barnegat Bay nesting beach sediments did not show an obvious POP contamination gradient but did provide insight on the atypical PBDE pattern

previously and currently reported in terrapin tissues and now measured in Barnegat Bay sediments. Our data suggest that Toms River, located in the northern part of the Bay, may be a source of PBDE 209 contamination and that the atypical pattern in terrapins might reflect accumulation of breakdown products of deca-BDE.

Introduction

Persistent organic pollutants (POPs) are anthropogenic chemicals that are resistant to degradation in the environment through chemical, biological and photolytic processes and are highly accumulative in wildlife and sediments throughout aquatic and terrestrial ecosystems (UNEP, 2009). All of the POPs discussed and reported in this paper are no longer produced or are restricted in production and use (UNEP, 2009). POPs, such as polychlorinated biphenyls (PCBs) and the chlorinated pesticides (OCPs), are called legacy compounds because even though they are no longer used they are still found in relatively high concentrations in wildlife and the environment. These compounds are monitored in the environment by sampling different compartments of a specific ecosystem such as sediment or wildlife. POPs accumulate in sediments due to their highly adsorptive physical properties (Jones and de Voogt, 1999). They attach to fine grained sediments and generally stay attached until they are redistributed to a place that has a higher affinity for the chemicals, such as lipids in organisms. Since POPs are lipophilic, they associate and accumulate in animal lipid stores (Jones and de Voogt, 1999). Once stored in animal tissues the contaminants may

be metabolized and redistributed within the body, causing toxic effects such as disrupted neurobehavioral development, reduced reproductive success, carcinogenicity and endocrine effects (Rattner et al., 2004; Kulkarni, 2005; De Solla et al., 2007; Darnerud, 2008).

Contaminants in an ecosystem are often monitored using an indicator species, because they represent the local contamination of an area as well as give possible information about toxicological risks associated with contaminants at environmental concentrations. Diamondback terrapins have been reported to be suitable indicators of organic contamination (Kannan et al., 1998; Blanvillain et al., 2007; Basile et al., Submitted). Terrapin plasma is an effective and relatively non-invasive monitoring tool for assessing POPs (Basile et al., Submitted). Reptile eggs are also often used as a tool to monitor POPs in the environment. For example, snapping turtle eggs are often used to monitor POPs in the Great Lakes Region (Bishop et al., 1998). Egg POP concentrations represent the levels of maternal POP contamination. Maternal transfer of lipophilic contaminants occurs during vitellogenesis when lipids and proteins are transferred from the maternal blood into the developing follicle as yolk (Holliday et al., 2009). Hence, compounds found in the egg track those accumulated by adult females from their foraging environment as well as the metabolites formed by the females (Alava et al., 2006). Maternal transfer has been documented in snapping turtles where significant correlations were observed between maternal tissues and eggs for PCBs and some OCPs, such as DDE, mirex and HCB (Pagano et al., 1999; Kelly

et al., 2008). Maternal transfer of PCBs, PBDEs and other OCPs has been suggested in diamondback terrapins (Basile et al., Submitted).

The primary objective of this study was to examine the POP concentrations in gravid female terrapins and their eggs to determine the occurrence and extent of maternal transfer of PCBs, PBDEs and OCPs in Barnegat Bay, New Jersey. Contamination profiles of known and suitable terrapin nesting beaches were also reported to determine if contamination of nesting sediments may serve as an important route of exposure to terrapins. The secondary objective of this study was to determine the spatial variation of POPs in terrapin tissue and nesting sediments along the shorelines of Barnegat Bay, NJ.

Materials and Methods

Study Site and Sample Collection

In June and July of 2008 15 female terrapins ranging from 605 g to 1438 g were captured on Great Bay Boulevard in Tuckerton, New Jersey. Great Bay Boulevard is 6.8 km in length and is the only road that travels through a peninsula of salt marsh in the southern most part of Barnegat Bay. Great Bay Boulevard is a site of high terrapin movement during the nesting season (Szerlag and McRobert, 2006). During high tide, females search for suitable nesting areas often on the shoulder of the road. The females were collected by hand from the road. Between five mL and eight mL of blood were drawn immediately after capture using the subcarapacial venipuncture method described elsewhere (Basile et al. Submitted). Terrapins were transported in individual five gallon buckets to

a field station in Waretown, NJ, where they were processed and induced to lay eggs. Terrapins were processed by taking morphometric measurements, notching individual alphabetical scute codes to the carapace and implanting passive integrated transponder (PIT) tags. After processing, these females were induced via interperotineal injection of 10 -30 IU/kg Oxytocin as described elsewhere (Sheridan et al., In Review). One egg from each of the 10 clutches collected was placed in a hexane-rinsed glass jar with a Teflon-coated lid and was frozen at -80 ^{o}C to provide background concentrations in eggs from maternal transfer. An additional four eggs per clutch were used for an incubation study, and the remaining eggs were placed in a hatchery and were released back to the Bay after hatching. Females were returned to the Bay close to where they were collected within 48 h after capture.

Barnegat Bay nesting beach sediments were collected from known or suitable nesting habitats for terrapins. One sample of about 85 to 125 grams was collected from each site and was placed in a hexane rinsed glass jar with Teflon-coated lid and was frozen at -80 ^{o}C.

Calibration Solutions and Quality Control

Seven calibration solutions were prepared at differing concentrations that were extracted and cleaned up alongside samples. Internal standard solutions were added to samples prior to extraction. The compounds in the calibration solutions and internal standard solution are described in detail in Chapter 2.

Three NIST SRMs were used as control samples. Three replicates of SRM 1957 (Organic Contaminants in Non-Fortified Human Serum) in addition to one replicate of an in-house control material of pooled loggerhead sea turtle plasma (Cc pool) were analyzed alongside the plasma samples. Three replicates of SRM 1947 (Lake Michigan Fish Tissue) were analyzed alongside the egg samples in addition to one replicate of an in-house control material of pooled loggerhead eggs (Cc comp). Three replicates of NIST SRM 1941b (Organics in Marine Sediment) were analyzed alongside the sediment samples. Laboratory procedural blanks and field blanks made from each lot of blood collection supplies were also analyzed.

Sample analysis

Plasma from the 15 live terrapins (between 2.7 g to 4.8 g) was extracted using focused microwave methods described in Chapter 2. A single pass of plasma extracts through an acidified silica column followed by an alumina column was sufficient. Eggs were mixed with diatomaceous earth, spiked with an internal standard solution of *iso*-octane, and extracted with DCM using pressurized fluid extraction (Dionex Corporation, Sunnyvale, CA) according to Keller et al. (2004a). These extracts were cleaned up using size exclusion chromatography with a 25 mm x 600 mm 10 um 100 Å PLGel column coupled to a 25 mm x 25 mm guard column (Polymer Laboratories) and also with acidified silica and alumina columns. Sediment samples were mixed with diatomaceous earth, spiked with an *iso*-octane internal standard solution, and extracted with

DCM using pressurized fluid extraction (Dionex Corporation, Sunnyvale, CA) according to Keller et al. (2004a). These extracts were cleaned up using size exclusion chromatography as described in Chapter 2 and also with acidified silica columns. Percent moisture was calculated for each sediment sample by measuring the mass of xx-xx grams of sediment in an aluminum weighing pan then placing the sediments in a drying oven at 120°C and recording loss of mass (water) every 24 hours for three days.

Lipid content was determined gravimetrically from initial extracts of plasma and eggs prior to any clean-up according to Keller et al. (2004a). Final extracts (≈ 0.2 mL in volume) were injected twice on a gas chromatograph mass spectrometer (GC/MS; Agilent Technologies 6890N/5973 inert, Palo Alto, CA) and the limit of detection (LOD) was determined as described in Chapter 2.

Statistical Analysis and Calculations

For descriptive statistics, values for non-detect samples were converted to half the limit of detection. All data were normally distributed. Before running statistics, concentrations <LOD for ΣPBDEs, ΣPCBs and 2, 4'-DDT, 4, 4'-DDE and 4, 4'-DDT were set to half LOD. The statistical program SAS version 8.1 (SAS Institute Inc., Cary, NC) was used to test correlations between plasma and egg concentrations and run One Way ANOVAs and Student-Newman-Keuls Test for site comparisons. Lipid normalized concentrations were used only for correlations.

Results and Discussion

Concentrations measured in all control materials were <10% different from certified values or past mean values for the majority of the compounds. The average percent differences for all reported values were -10.3 %, 4.7 %, -1.6 %, -0.03 % and 4.1 % for SRM 1947, Cc Comp, SRM 1957, Cc Pool and SRM 1941b, respectively. Precision was assessed by comparing the relative standard deviations of three replicates each of SRM 1947, 1957 and 1941b and they were on average 2.5%, 11.6 % and 18.5% respectively.

Contaminant Concentrations and Patterns in Terrapin Plasma

In female terrapin plasma the predominant contaminants were ΣPCBs, followed by 4, 4'-DDE, oxychlordane and ΣPBDEs (Table 1). Only one other pesticide, mirex, was detected in the plasma samples (Table 1). The concentrations of the summed contaminant classes as well as the pesticides are similar to those measured previously in terrapins from Barnegat Bay (Basile et al., Submitted). The detection of these contaminants and their concentrations are also similar to other marine and freshwater turtle species (de Solla and Fernie, 2004; Keller et al., 2004a; Moss et al., 2009).

The top five predominant PCB congeners accounted for 71.6% of the total PCBs. The most predominant congener was PCB153+132 (36%) followed by PCBs 118, 138, 180+193 and 99 (Figure 2). Similar patterns of PCB congeners, meaning same predominant congeners although not always in the same order, have been reported in female and male terrapin plasma as well as in the plasma of

other freshwater and marine turtle species (de Solla and Fernie, 2004; Keller et al., 2004a; Moss et al., 2009; Basile et al., Submitted).

An atypical PBDE congener pattern was seen in the gravid female terrapin plasma. The top 4 predominant congeners that were detectable in at least half the samples were PBDE 154 (74%) followed by PBDE 100, 153, and 183. The top 4 congeners account for 98% of the total PBDEs (Figure 3). A similar atypical profile was seen in a previous terrapin study from Barnegat Bay (Basile et al., Submitted). The typical PBDE pattern seen in most other wildlife has PBDE 47 and 99 as the most predominant congeners followed by PBDEs 100, 153 and 154 (Hites, 2004). The atypical pattern of the hexa brominated compounds (153, 154) occurring in larger proportions compared to the tetra and penta-brominated compounds (47,99) has been reported in other freshwater and marine turtle species but is not specific to all turtle species (Keller et al., 2005; Hermanussen et al., 2008; Moss et al., 2009).

Contaminant Concentrations and Patterns in Terrapin Eggs

Eleven of the 15 terrapins captured laid their clutches. Clutch sizes ranged from 8 to 15 eggs with an average clutch size of 11 eggs. Similar contaminants were detected in terrapin eggs as compared to the plasma, but more pesticides were measured above the limit of detection in the eggs (Table 1). Higher concentrations and additional detections were expected in eggs due to the greater amount of lipid present than in plasma. The predominant contaminants were ΣPCBs, followed by 4, 4'-DDE, oxychlordane then ΣPBDEs. Also detectable

were mirex, HCB and two other metabolites of DDT (Table 1). Similar compounds were detected in terrapin eggs from a previous study (Basile et al., Submitted). In our previous terrapin study the mean contaminant concentrations only represent one full clutch whereas in this study the measurements represent 10 clutches (one egg per clutch analyzed).

Terrapin egg POP concentrations are within similar ranges compared to other turtle species. When compared to snapping turtle eggs from two reference sites from Lake Erie, terrapins had almost double the concentration of ΣPCBs and 4, 4'-DDE but were in the same range for oxychlordane, mirex and HCB (de Solla and Fernie, 2004). Terrapin eggs were less contaminated in comparison to loggerhead sea turtle eggs collected from beaches in the southeastern United States (Alava et al., 2006). Loggerhead eggs had twice the ΣPCB concentration found in terrapin eggs from this study. Loggerhead eggs from North Carolina have three times the ΣPBDEs compared to terrapin eggs from this study (Keller et al., 2005). Loggerhead and leatherback sea turtle eggs from Florida however have similar ΣPBDE concentrations compared to these terrapin eggs (Keller et al., 2005).

The top five predominant PCB congeners in terrapin eggs were PCB 153+132 (34%), followed by PCBs 118, 138, 99 and 180+193 (Figure 2). The top five congeners accounted for 67.8% of the total PCBs. This pattern is very similar to that seen in the terrapin plasma from this study except for plasma PCB 180+193 which was more predominant than PCB 99. Similarity was expected

because PCBs are assumed to maternally transfer, so the egg patterns should reflect those of maternal tissues. Other studies have observed maternal transfer of PCBs in turtles and the data suggest that there is not preferential transfer of higher or lower chlorinated PCBs from female to offspring (Dabrowska et al., 2006; Kelly et al., 2008).

An atypical PBDE pattern was also seen in the eggs. The top five predominant congeners were, PBDE 153 (20%) followed by PBDE 154, 47, 155 and 99 (Figure 3). The top five congeners accounted for 80.7% of the total PBDEs. The predominance of the hexa-brominated congeners in this study were similar to the clutch measured in our previous terrapin study (Basile et al., Submitted). There are two major differences between this study and the previous study. First is the absence of PBDE 100 in the current study. It was the most predominant PBDE in the clutch from the previous study. Second is the detection of PBDE 47 in the current study and that it is the third most predominant congener. PBDE 47 was not detected in the clutch from the previous study. Both discrepancies likely have to do with the fact that the previous study only represents one clutch from a different sampling area whereas the current study represents 10 clutches from one single sampling area and shows more variation within the population.

Maternal Transport

We performed correlations to determine if egg POP concentrations can be predicted by female plasma concentrations. We did not find any significant

relationships for POP concentrations between the gravid female plasma and her eggs. All correlation coefficients were less than 0.32. This was an unexpected result because maternal transport in terrapins has been validated through the presence of POPs in eggs that were both directly dissected from a female as well as taken directly after ovoposition (Basile et al., Submitted) and current study). In other reptile species significant positive correlations for total PBCs and OCPs were observed between female plasma and eggs in snapping turtles and American alligators (Rauschenberger et al., 2004; Dabrowska et al., 2006). There are also reptile studies where no significant correlations are seen between contaminant concentrations in female plasma and egg. Kelly *et al.* report female snapping turtles collected from a contaminated site had significantly correlated PCB concentrations but those collected from the reference sites did not (2008).

There are two schools of thought when determining the source of lipophilic contaminants in eggs. First, contaminants in eggs are derived from contaminants stored in maternal somatic lipids, which are long-term storage depots. Second, contaminants are associated with the short-term storage lipids derived from diet recently consumed by the female circulating in her plasma. There are studies suggesting either one or a combination of both sources of POPs to the eggs (Bishop et al., 1994; Pagano et al., 1999; Rauschenberger et al., 2004; Dabrowska et al., 2006).

The determination of whether terrapin egg POP concentrations are derived from maternal somatic lipid stores or the lipids of recent maternal diet can be

examined by studying the reproductive cycle of the terrapin. Terrapins are reported to have a post-nuptial reproductive system where females conduct the majority of follicular development in the late summer and fall before brumation (Lee, 2003). In painted turtles, who exhibit similar seasons of follicular development, the energy input for the initial development of follicles comes from recently harvested food sources, whereas post-hibernation the energy input comes from lipid stores (Congdon and Gibbons, 1990). If terrapin energetic investments towards follicular development are similar to painted turtles then plasma samples collected directly before ovoposition may no longer reflect the diet of the terrapin in the previous late summer and fall when the majority of the follicular development occurred. In a chemical equilibrium model for estimating the maternal transfer of organochlorines in oviparous organisms, eggs receive contaminant concentrations that resemble maternal tissues rather than prey items because the dietary lipids rapidly adopt the same lipid based contamination as somatic lipids (Russell et al., 1999). In a previous terrapin study POP concentrations were significantly and positively correlated between plasma and fat (Basile et al., Submitted). If the dietary lipids terrapins consume theoretically are to rapidly assume the contaminant patterns in terrapin somatic tissue as suggested by the plasma fat correlations and the model introduced by Russell et al. then we would expect significant correlations between terrapin plasma and eggs (1999). Because we did not see these correlations we assume that in terrapins, dietary lipids may not assume the contaminant patterns in terrapin

somatic tissue as rapidly therefore the non-significant correlations seen between terrapin plasma and eggs is due to a time lag between follicle development and egg laying. We would then expect that collecting blood at the end of nesting season prior to brumation and comparing that to eggs laid in the spring would show significant correlations.

Contaminant Concentrations and Patterns in Sediments

The predominant POPs found in nesting beach sediments in Barnegat Bay were PCBs, 4, 4'-DDE, PBDEs and 2, 4'-DDT (Table 2). PCB concentrations in Barnegat Bay nesting sediments which ranged from 0.175 to 65.5 ng/g dry mass were on the lower end of the range when compared to sediment samples from sites along the Delaware River Estuary in Pennsylvania and Delaware where total PCBs range from 30 to 216 ng/g dry weight and were similar to Great Lakes, Lakes Erie and Ontario, where total PCBs range from 23 to 63.6 ng/g dry weight (Table 2; Song et al., 2005; Ashley et al., 2007). Barnegat Bay PBDE concentrations which ranged from 0.399 to 7.65 ng/g dry mass were similar to those seen in Lake Erie and Ontario which ranged from 1.95 to 6.33 ng/g dry mass however they were much lower than those seen in sites from PA and DE which range from 0.73 to 21.7 ng/g dry mass (Table 2; Song et al., 2005; Ashley et al., 2007). The two pesticides that were detectable in Barnegat Bay sediment are also common in other sediment and wildlife (Table 2). Nesting beach sediment had greater concentrations of 4,4'-DDE and 2,4'-DDT when compared to sediment from Barnegat Inlet sampled in 1996, which had concentrations of

1.31 and 0.32 ng/g dry weight of 4,4'-DDE and 2,4'-DDT respectively (Table 2; NCCOS, 2010). This increased concentration in nesting beach sediment may be due to several factors. One may be a temporal difference. POPs can accumulate over time with ongoing inputs of contamination. Second, the location of sampling may explain differences. In the current study, samples were collected from nesting beaches on the western coast of the bay whereas Barnegat Inlet is on the eastern coast and may be influenced by ocean water. Lastly, the type of sediment may account for differences. Because the inlet is in close contact with the ocean it is washed regularly by ocean water and is mostly sand where as the nesting beach sediment will most likely have greater concentrations of organic matter that is known to hold contaminants.

It is important to note at this point that the nesting beach sediment samples collected for this study were collected from land that is occasionally washed with bay water and is more likely to receive precipitation and atmospheric deposition of contaminants, whereas most studies reporting contaminants in sediments acquire their sediment from the benthic environment, which is completely submerged under water and may be subject to differing routes of exposure. Differing routes of exposure may reflect different patterns of accumulation.

The PCB congener pattern found in Barnegat Bay is predominated by PCBs 138 (37%), 95+121 and 105, 110 and 153+132 (Figure 4). The top five predominant congeners represent 61.7 % of the total PCB contamination. There were less individual PCB congeners found in sediments compared to terrapin

plasma and eggs. Barnegat Bay nesting beach sediments seem to be predominated by moderately chlorinated congeners. The common congeners seen in Lake Erie and Ontario superficial sediments seem to be mostly lower and some middle chlorinated congeners (Song et al., 2005). In the NIST SRM 1944, marine sediment from the mouth of the Baltimore Harbor PCB pattern was dominated by mostly lower chlorinated compounds (May and Watters, 2004). In the NIST SRM 1941b, sediment collected from New York and New Jersey Waterway, PCB congener pattern was dominated by lower to mid chlorinated PCBs (Wise and Watters, 2008). The congeners Barnegat Bay had in common with the Great Lakes was PCBs 153, 180 and 187 and with the SRMs, PCBs 153 and 110 (May and Watters, 2004; Song et al., 2005; Wise and Watters, 2008). In general Barnegat Bay nesting beach sediments have both similar and dissimilar PCB congener patterns in comparison to other reported sediment samples. This is most likely due to the high variability often seen with sediment samples and the location, land versus submerged, in which the sediment was collected.

Barnegat Bay nesting beach sediments had an atypical PBDE congener pattern similar to what was seen in terrapins from the current and previous study (Basile et al., Submitted). The top five predominant congeners were PBDE 209 (55.6%) followed by 138, 181, 156 and 154 (Figure 4). These five congeners accounted for 98.4% of the total PBDEs in sediments. Typically PBDE patterns in sediments are predominated by PBDE 209, then follow a similar pattern seen in wildlife where PBDEs 47 and 99 are in greater proportions in comparison to

PBDEs 100, 153 and 154 (Hites, 2004). The typical pattern was observed in sediments along the Delaware River Estuary and SRMs (Ashley et al., 2007). The origin of this atypical pattern in Barnegat Bay is unknown. The sediment congeners are not similar to congener compositions found in penta, or octa-technical mixtures (La Guardia et al., 2006). It is possible that the PBDE contamination of Barnegat Bay originated from deca-PBDE, and the unexpected congeners may be breakdown products of PBDE 209, which is the primary congener in deca-PBDE technical mixture. There is a fabric coating mill located in Ocean County and is specifically located within the Toms River watershed. Toms River is a tributary that has a large input of surface water into Barnegat Bay. They have reported the use and the associated waste which totals about 700 kilograms of deca-BDE in their facility from 2006 to 2008 (USEPA, 2006-2008). Although the waste is reported to be disposed of offsite the fact that it is used provides possible sources of deca-BDE through atmospheric transport and possible run off in and around the facility using the product. The breakdown products from deca-BDE are most likely from debromination. There are studies suggesting debromination of PBDE 209 can occur through anaerobic, photolytic, and microbial reductive pathways (Soderstrom et al., 2004; Gerecke et al., 2005; He et al., 2006). Debromination and hydroxylation of PBDE 209 has also been reported *in vivo* as well as in the plant soil system (Stapleton et al., 2004b; La Guardia et al., 2007; Huang et al., 2010).

Geographic Comparisons: Tissues and Nesting Beaches

In a previous study we reported significant differences in POP concentrations in terrapin tissues between two sites (Forsythe National Wildlife Refuge and Spizzle Creek on Island Beach State Park) that were separated by10 km (Basile et al., Submitted). The current study expanded the geographical comparison to a third site within Barnegat Bay, Great Bay Boulevard, which is approximately 32 km south of the previously sampled sites (Figure 1). The female plasma concentrations combined from the two northern sites (Forsythe/Spizzle, n=8) were significantly higher for ΣPCBs and 4, 4'-DDE compared to Great Bay Boulevard (n=15) (Figure 5). There was not a significant difference for ΣPBDEs or oxychlordane even though oxychlordane does appear to have greater concentrations in Forsythe/Spizzle terrapin plasma (Figure 5). These significant differences reinforce the idea that northern portions of Barnegat Bay are more contaminated with POPs than the southern portion. We expect higher concentrations in the northern sites due to greater industrial and human population density in the northern portion of the bay and its watershed. Because 70% of the inflow into the bay from its watershed enters the bay above Barnegat Inlet we expected a decreasing concentration gradient from north to south through the bay. Similar results were seen in Biscayne Bay, FL bottlenose dolphin tissue, another species that can be used to monitor POPs in the estuarine/marine environment (Irwin, 2005). Resident populations of dolphins from the more urbanized northern portion of the bay had greater POP concentrations compared to the

resident dolphins from the rural southern portion of the bay (Litz et al., 2007). Similarly in terrapins, the individuals with the greatest POP concentrations were associated with the industrialized and more urbanized region of Barnegat Bay. There are confounding factors such as the season of sampling as well as the differing ages/size of animals and the difference of years in sampling that should be considered. We do not believe these factors had a large influence on the concentrations in POPs measured in their tissue because all females had similar ranges in mass between the two sampling years and sites. There may be an influence on season of capture. Females from the northern sites were captured in September while yolking and feeding whereas the females from the southern site were nesting and most likely fasting so we would expect the northern females to have slightly higher levels of circulating POPs in their plasma based on season of capture. However the females sampled in the southern site were captured in 2008 whereas the northern females were captured in 2006 so we might expect the southern females to have had a chance to accumulate more POPs.

Barnegat Bay nesting beach sediments showed no obvious spatial pattern in POP concentrations. The sites were chosen because they were either known or assumed suitable nesting beaches for terrapins . All were located on the western coast of the bay and represented the entire length of the bay (Figure 1.) We expected the same north-south contamination gradient in the nesting beach sediment as seen in terrapin tissues, but this was not observed. The results may be due to the inherent high variability often observed in sediments. Because of

time and funding constraints, only one sample was measured at each site. Standard sediment sampling protocols use multiple sediment samples taken from a site which are then homogenized before extraction. This helps account for the small scale spatial variability which tends to be large in sediments (Strobel and Schimmel, 1991; Collins, 1995; Heitmuller, 2001). Although no gradient was seen, some interesting findings came from this small sample set of sediments. From north to south, PBDE concentrations are low until directly south of Toms River and then they remain greater than those above Toms River (Figure 1). These data suggest there is an input of PBDEs into Barnegat Bay from that particular part of the watershed. As mentioned in the previous section there may be a direct influence of deca-BDE to the watershed from a facility in the Toms River watershed that uses deca-BDE in the production of their textiles (USEPA, 2006-2008). Additionally, one sampling site directly north of Great Bay Blvd. had high concentrations of all three classes of POPs (Figure 1). The sediment from this site was different from the others as it was taken from directly behind a bulkhead, which may have retained POPs in this site whereas the other sites could be better flushed during rain events and direct contact with the Bay water. The levels of POPs measured in nesting beach sediments from this study would be used better as a representation of the contamination patterns found in Barnegat Bay as a whole rather than each site specifically.

Conclusions

Although no significant correlations were seen between maternal POP plasma concentrations and egg POP concentrations, maternal transport was demonstrated simply by the presence of POPs in eggs that had never touched sediments. Based on terrapin reproduction and energy investments in follicular development, a more descriptive picture of maternal transfer of POPs may be seen between eggs and maternal somatic tissues at the time of sampling. The examination of POP concentrations in terrapin nesting beach sediments showed an interesting contamination pattern for sum PBDEs, where PBDE concentrations were low in the northern sites sampled above Toms River but increased just below Toms River and stayed elevated in the rest of the southern sampling sites. The data suggest an input from the Toms River watershed. A textile coating plant that uses deca-PBDEs in Toms River watershed may explain the atypical PBDE pattern reported in terrapin tissues and sediments. The significant differences in POP contamination in terrapin plasma between sites further strengthens the suitability of using terrapins as indicators of local POP contamination in estuarine ecosystems.

Table 1. Summary of persistent organic pollutant concentrations (ng/g wet mass) in plasma of 15 gravid female terrapins and 10 terrapin eggs from Great Bay Blvd, Barnegat Bay, NJ. Only predominant contaminants and congeners are listed.

	Terrapin Plasma ng/g wm n=15						Terrapin Eggs ng/g wm n=10					
Compound	**Mean**	**Stdev**	**Median**	**Min**	**Max**	**n>LOD**	**Mean**	**Stdev**	**Median**	**Min**	**Max**	**n>LOD**
HCB	<LOD	<LOD	<LOD	<LOD	<LOD	0	0.050	0.015	0.051	0.029	0.081	10
Oxychlordane	0.153	0.078	0.146	0.046	0.270	15	3.68	2.01	3.55	1.26	8.01	10
Mirex	0.068	0.085	0.003	<LOD	0.246	7	0.232	0.133	0.194	0.098	0.449	10
4,4'-DDE	0.397	0.210	0.423	0.137	0.755	15	8.17	3.76	7.35	3.38	13.8	10
4,4'-DDD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0.060	0.022	0.056	0.029	0.100	10
4,4'-DDT	<LOD	<LOD	<LOD	<LOD	<LOD	0	0.018	0.015	0.014	<LOD	0.046	8
PCB 99	0.384	0.267	0.271	0.155	1.07	15	5.33	2.13	5.30	2.34	8.63	10
PCB 118	0.732	0.524	0.440	0.311	2.02	15	9.26	4.45	8.01	3.12	18.6	10
PCB 138	0.648	0.408	0.427	0.259	1.47	15	6.15	2.80	5.96	2.37	11.8	10
PCB 153+132	2.24	1.66	1.34	0.936	6.91	15	24.7	12.8	21.1	11.0	54.9	10
PCB 180+193	0.432	0.301	0.353	0.151	1.34	15	3.91	2.65	2.80	1.60	10.6	10
ΣPCBs	6.23	4.38	3.70	2.53	17.7	15	72.4	34.8	61.4	31.3	150	10
PBDE 47	<LOD	<LOD	<LOD	<LOD	<LOD	0	0.103	0.124	0.064	0.042	0.453	10
PBDE 99	<LOD	<LOD	<LOD	<LOD	<LOD	0	0.072	0.040	0.065	0.024	0.162	10
PBDE 100	0.016	0.020	0.013	<LOD	0.061	8	<LOD	<LOD	<LOD	<LOD	<LOD	0
PBDE 154	0.051	0.034	0.036	0.019	0.125	15	0.104	0.027	0.103	0.066	0.166	10
PBDE 153	0.009	0.009	0.010	<LOD	0.025	9	0.123	0.073	0.100	0.047	0.309	10
PBDE 183	0.012	0.025	0.003	<LOD	0.089	2	0.049	0.043	0.037	<LOD	0.129	7
PBDE 209	<LOD	<LOD	<LOD	<LOD	<LOD	0	<LOD	<LOD	<LOD	<LOD	<LOD	0
ΣPBDEs	0.088	0.082	0.056	0.019	0.300	15	0.608	0.253	0.576	0.260	1.130	10
% Lipid	0.447	0.193	0.38798	0.186	0.815	15	6.05	1.40	6.36	2.93	8.26	10

<LOD = below the limit of detection

Table 2. Summary of persistent organic pollutant concentrations in sediment from 10 nesting beaches. Only predominant contaminants and congeners are listed and concentrations are listed in ng/g dry mass.

Terrapin Nesting Beach Sediment ng/g dry mass

n = 10

Compound	Mean	Stdev	Median	Min	Max	n>LOD
4,4'-DDE	8.45	17.0	0.103	<LOD	53.9	4
2,4'-DDT	0.753	1.11	0.249	<LOD	3.54	5
4,4'_DDT	32.6	95.2	0.133	<LOD	303	3
ΣDDTs	55.9	156	0.952	<LOD	500	6
PCB 110	0.646	1.43	0.020	<LOD	4.63	4
PCB 138	0.787	1.40	0.264	<LOD	4.55	8
PCB 149	0.645	1.55	0.019	<LOD	4.95	3
PCB 153+132	1.12	2.49	0.035	<LOD	7.77	3
PCB 180+193	0.785	1.72	0.029	<LOD	5.30	3
ΣPCBs	9.5	20.7	0.40	0.174	65.5	10
PBDE 138	0.250	0.117	0.281	0.115	0.409	10
PBDE 154	0.186	0.275	0.023	<LOD	0.624	3
PBDE 156	0.133	0.080	0.137	<LOD	0.231	9
PBDE 181	0.106	0.048	0.120	0.049	0.179	10
PBDE 209	1.99	2.16	1.14	<LOD	6.29	7
ΣPBDEs	2.66	2.49	1.46	0.399	7.65	10

<LOD = below the limit of detection

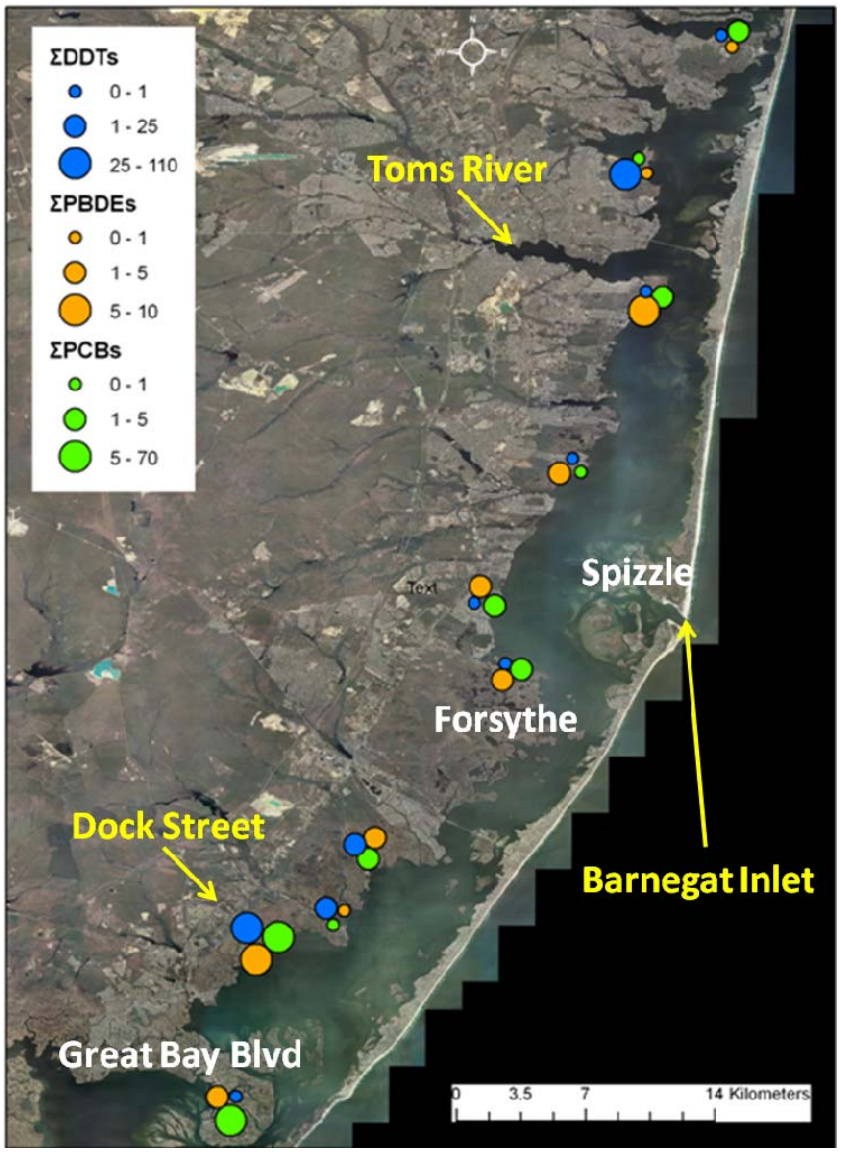

Figure 1. Map of ten nesting beach sediment persistent organic pollutant concentrations and three female plasma terrapin sampling sites in Barnegat Bay, NJ. POP concentration in ng/g dry mass. Terrapin sampling sites are labeled white, places of interest are labeled yellow.

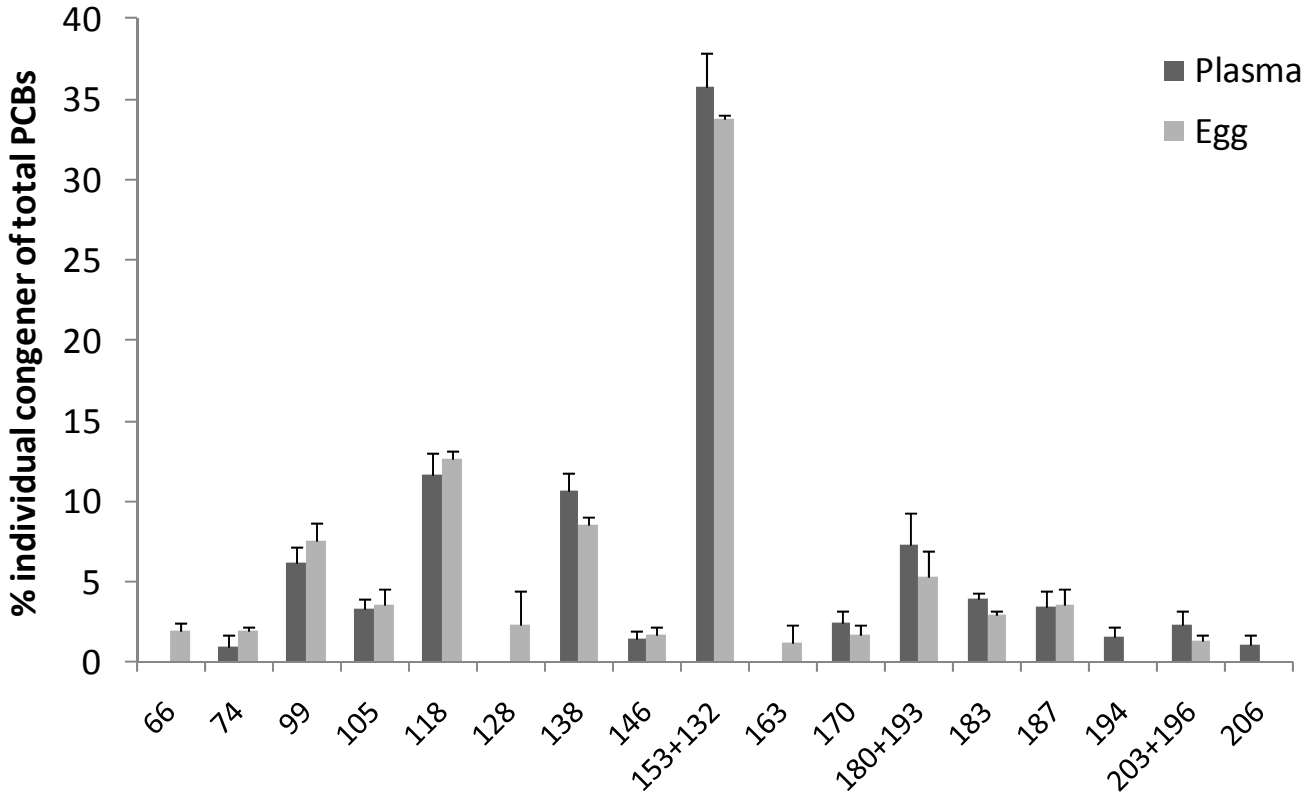

Figure 2. PCB congener patterns in gravid female terrapin plasma and eggs. Congeners listed are those above 1% of total PCBs. Error bars represent standard deviation.

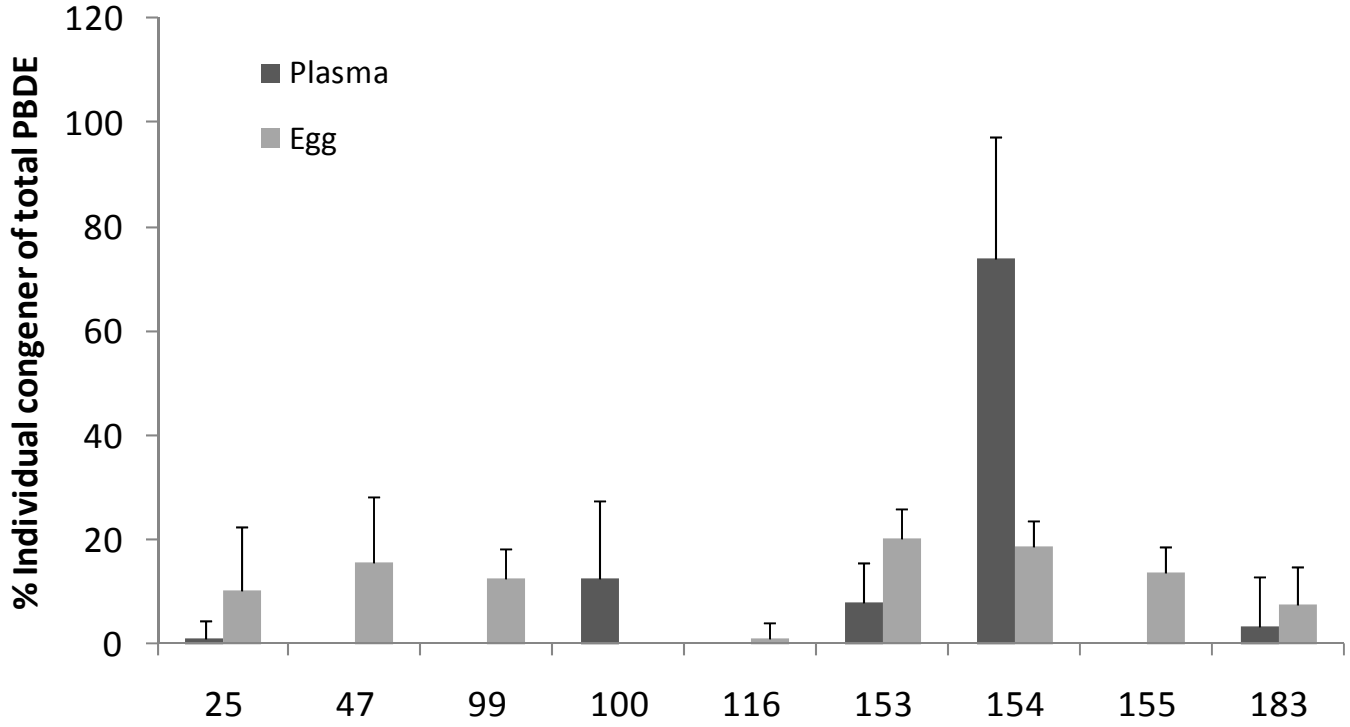

Figure 3 PBDE congener patterns in gravid female terrapin plasma and eggs. Congeners listed are those above 1% of total PBDEs. Error bars represent standard deviation.

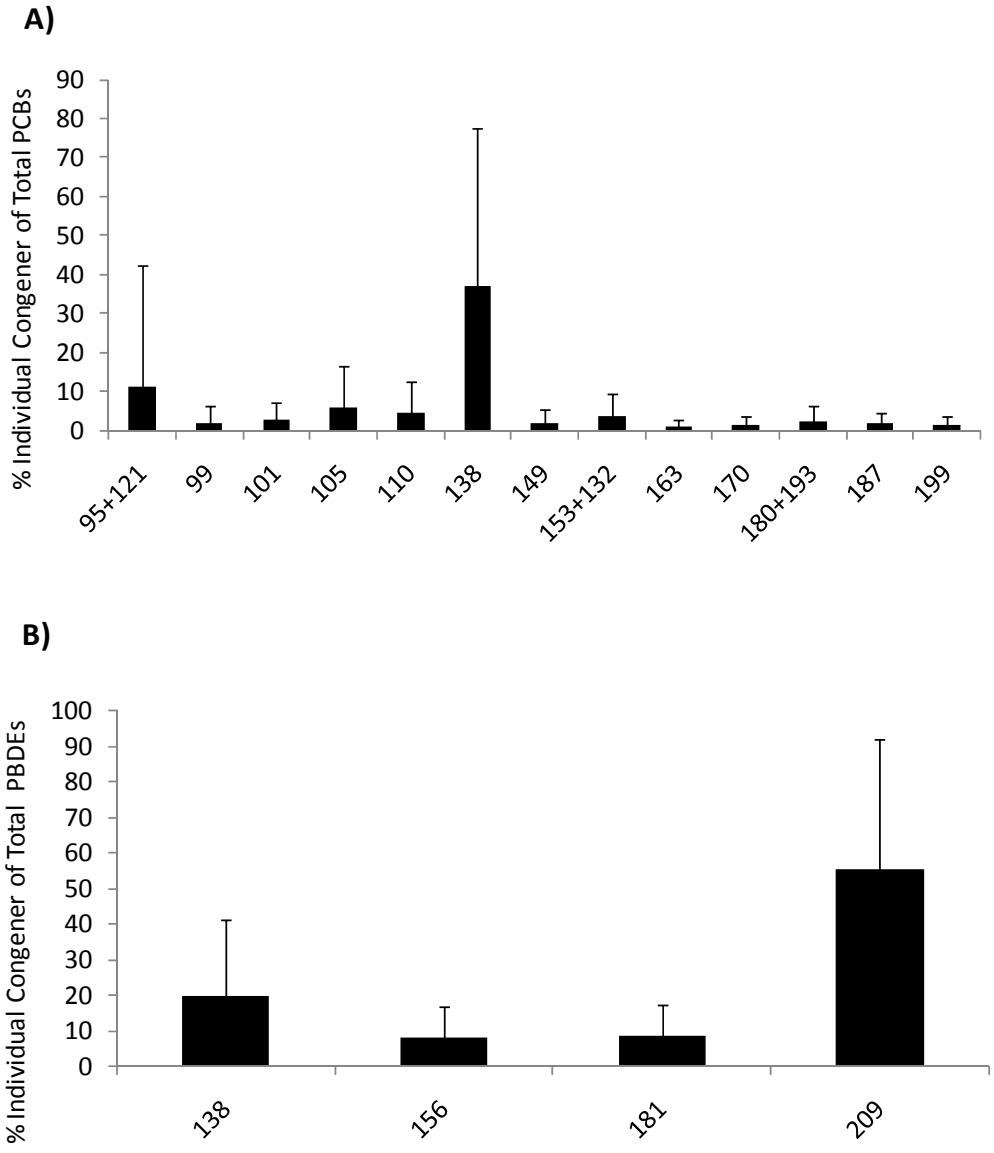

Figure 4. A and B. A) PCB and B) PBDE congener patterns in Barnegat Bay Nesting Sediments. Congeners listed are those above 1% of total and in at least 30% of the samples. Error bars represent standard deviation.

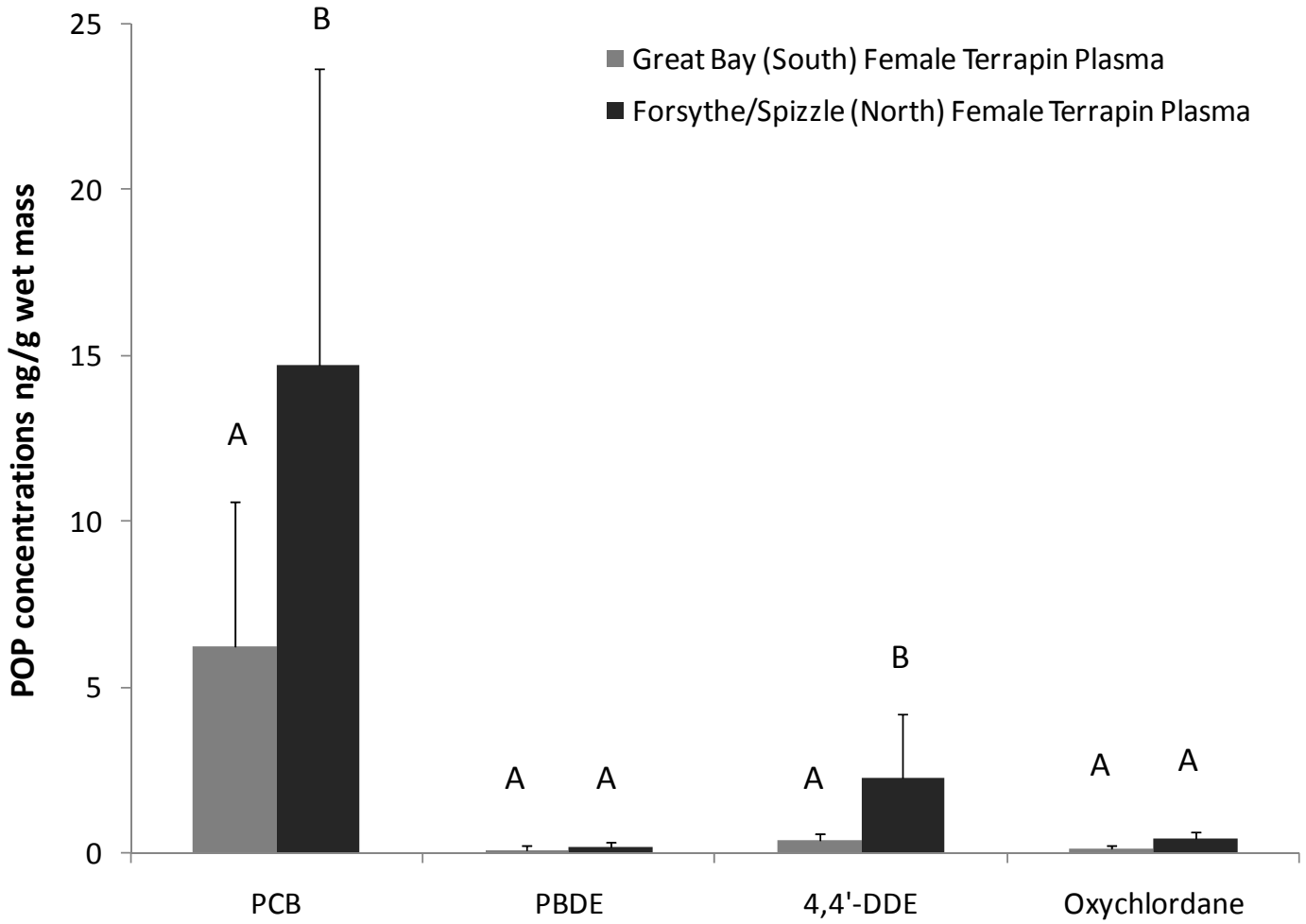

Figure 5. Persistent organic pollutant concentrations (ng/g wet mass) in female diamondback terrapin plasma from two regions of Barnegat Bay, NJ.

CHAPTER 4:
TRANSFER OF POLYBROMINATED DIPHENYL ETHERS (PBDEs) AND OTHER PERSISTENT ORGANIC POLLUTANTS (POPs) FROM NESTING SEDIMENTS TO DEVELOPING DIAMONDBACK TERRAPIN EGGS

Abstract

Persistent organic pollutants (POPs) are ubiquitous in the environment and wildlife. The presence of POPs in eggs and hatchlings are generally attributed to maternal transfer of POPs during yolking and follicle development. Another less studied route of exposure to developing embryos is the sediment in which they are incubating. Diamondback terrapin eggs were incubated in treatment sediments prepared by spiking sand and dredged sediments collected from Barnegat Bay, NJ with a 1:10 ratio of DE-71 (penta-BDE technical mixture) and Saytex 102-E (deca-BDE technical mixture). Mean ΣPBDE concentrations measured for spiked sediments were 13.4 μg/g and 14.6 μg/g for spiked sand and spiked dredge, respectively. PBDE transfer ranging from 0.0001 to 1.7% was observed from spiked and non-spiked sand and dredged sediments to terrapin eggs. Our goal of determining which sediment type, sand or dredged sediment, would facilitate the greatest transfer was not attainable due to the lack of development in the dredged sediments. We did observe significantly greater transfer in developing terrapin eggs. There were no obvious fatal deformities or decreased developmental success of eggs/embryos in the spiked sand compared to the non-spiked sand.

There was however a higher occurrence of carapace vertebral and marginal scute anomalies in the PBDE spiked sand. Based on the transfer of PBDEs to the eggs incubated in non-spiked sand (0.256 – 3.14 ng/g), which represents a somewhat underestimated potential of transfer in environmental conditions, and the PBDE concentrations measured in ten Barnegat Bay nesting beaches we can assume that PBDE transfer in natural nesting beaches is negligible at this site and that the major route of exposure to developing terrapin eggs is through maternal transfer. The transfer of other POPs was also measured. Polychlorinated biphenyls (PCBs), mirex, and metabolites of DDT showed transfer ranging from 0.0009 to 2.2% from sediment to developing embryos, with PCB 180+193 showing the greatest transfer of all POPs excluding PBDEs.

Introduction

Persistent organic pollutants (POPs) are ubiquitous in the environment. In context to a female diamondback terrapin, POPs are found in the tissues of the reptile, its prey items as well as its environment, including nesting beaches. POPs, like polychlorinated biphenyls (PCBs) and the chlorinated pesticides (OCPs), are commonly called legacy compounds because even though they are no longer used they are still found in relatively high concentrations in wildlife and the environment. Polybrominated diphenyl ethers (PBDEs), used as additive flame retardants in electrical components and upholstery are however still in production (Darnerud et al., 2001). Although they are restricted, one of the three most produced commercial mixtures, deca-BDE, is still in use in the United States.

Deca-BDE is 97% composed of PBDE 209 which is a congener that was originally thought to be relatively non-bioavailable (Darnerud et al., 2001; La Guardia et al., 2006). This however is a disputed topic because PBDE 209 has been measured in biological samples but more importantly is known to be unstable in the environment and can debrominate into lower brominated compounds that are more bioavailable and toxic to wildlife (Soderstrom et al., 2004; Stapleton et al., 2004b; Lee and He, 2010).

The omnipresence of POPs suggests many possible exposure routes for these contaminants to enter terrapin tissues. The route of dietary exposure to POPs through bioaccumulation and biomagnification has been well established in aquatic and marine food webs (Borga et al., 2001; Stapleton and Baker, 2003; Burreau et al., 2006). The route to developing offspring has been attributed to maternal transfer through vitellogenesis, in reptiles (Holliday et al., 2008). Exposure to these contaminants at crucial points of early development can cause permanent damage to reproductive, endocrine and immune systems and neurobehavioral development (Guillette et al., 1995; Willingham and Crews, 1999; Kuriyama et al., 2005). Many of these effects are somewhat unnoticeable because they occur internally. Other effects such as decreased egg survival and increasing severity of deformities have been reported to occur at increasing concentrations of POPs (Bishop et al., 1991; Van Meter et al., 2006).

The presence of POPs in the environment, specifically in soils and sediments available for terrapin nesting, has the potential to introduce more contamination to

developing eggs if they do indeed transfer into the egg during incubation. Organic contaminants are known to have high affinities for sediment particles because of their hydrophobicity. The strength of organic contaminant absorption to sediment particles is correlated to the amount of organic carbon associated with the sediment, its clay type and content, cation exchange capacity and pH (Knezovich et al., 1987). Dredged sediment in Barnegat Bay, New Jersey, which was sediment that originated from a submerged benthic environment, generally has finer particles and more clay and organic matter when compared to the sand commonly found on natural nesting beaches (Wnek et al., In Review). Because Barnegat Bay is so shallow, it is constantly dredged and in the past as well as currently the dredged sediment is placed on the shore making viable nesting areas for terrapins and other nesting species of the bay. Due to the characteristics of dredged sediment, higher concentrations of POPs may be available to developing eggs compared to eggs developing in natural sandy nesting sediments.

The major goals for this study were to measure the amount of transfer of PBDEs from incubation sediments to developing terrapin eggs and to determine which sediment type, natural (sand) or dredged sediment facilitated the greatest transfer then compare results to the terrapins true nesting environment. Secondary goals included determining effects to the embryo due to transfer of PBDEs in the PBDE spiked sediments and measuring transfer of other POPs from incubation sediments to developing terrapin eggs.

Materials and Methods

Study Site and Sample Collection

In June and July of 2008, 15 female terrapins ranging from 605 to 1438 grams were captured on Great Bay Boulevard in Barnegat Bay, New Jersey. Females were collected by hand off of the road. Between five mL and eight mL of blood were drawn immediately after capture using the subcarapacial venipuncture method described elsewhere (Basile et al., in Prep). They were placed into their own five gallon bucket then transported back to our field station in Waretown, NJ where they were processed and induced to lay eggs. Terrapin processing included taking morphometric measurements, being given individual alphabetical scute codes and being implanted with a passive integrated transponder (PIT) tag. After processing, these females were induced via interperotineal injection of oxytocin as described elsewhere (Sheridan et al., In Review). Eleven of 15 terrapins laid their clutches. Clutch sizes ranged from 8 to 15 eggs with average clutch size of 11 eggs. All eggs were weighed and measured. One egg from 10 of these clutches was placed in a hexane-rinsed glass jar with a Teflon-coated lid and was frozen at -80 ^{o}C. An additional four eggs per clutch were used for the incubation study described here, and the remaining eggs were placed in a hatchery where they were released back to the bay after hatching. The eggs collected for the incubation study were transported back to Drexel University in Philadelphia, PA and were placed into incubation sediments.

Female were returned to the water closest to the place where they were picked up off the road no more than 48 hours after capture.

The sediments used for this study were collected from nesting areas of terrapins in July of 2008. The sand "natural" sediment was collected from a nesting beach located on the Edwin B. Forsythe National Wildlife Refuge and the dredged sediment was collected from a pile of fresh dredge (less than 6 months old) that originated in the boat channel alongside of the field station in Waretown, NJ. Sediments were also collected from ten nesting beaches that spanned the west coast of Barnegat Bay for comparison to natural nesting beach contaminants and concentrations.

Incubation Sediment Spiking Design

One of four eggs collected from each clutch was randomly placed in one of four incubation treatments; sand, dredge, PBDE spiked sand or PBDE spiked dredge. The spiking solution was prepared by mixing two commercial PBDE mixtures, DE-71 (Lot# I1-9570) and Saytex 102E (Lot# I1-6247) purchased from Cambridge Isotope Laboratories Inc. at a 1:10 ratio respectively in hexane (90 ml), dichloromethane (2.5 ml) and toluene (372.5 ml). The ratio of spiking solution was based on the composition and concentrations of major PBDEs found in NIST SRM's 1941b and 1944, both marine sediment. To determine the ng/g of PBDE needed in the sediment to be able to measure transfer into eggs we used the average ΣPBDE concentration in a clutch of 11 terrapin eggs from a previous study, which was 1.7ng/g (Basile et al. In Progress). Because this was an average

we doubled the concentration to an estimated 3.4 ng/g to conservatively estimate the maximum PBDE concentration in terrapin eggs. We estimated that one gram of sediment will be available to transfer PBDEs to the egg which was considered a reasonable estimated mass of sediment touching and surrounding the egg while incubating. We expected less than 10% to transfer into the egg (Podreka et al., 1998). Due to the already low PBDE concentrations in terrapin eggs and the GCMS's limits of detection we needed to change the egg PBDE concentration substantially to detect a difference. We expected to be able to detect a difference if there is at least 8 ng PBDE greater than the original egg contents. All sediments were baked over night at about 165°C (Precision Gravity Convection Oven, model #18EG) before spiking. About 4700 grams of oven dried sand were spiked with 11.5 mg DE-71 and 92.6 mg Saytex 102E and about 4100 grams of oven dried dredged sediment were spiked with 11.6 mg of DE-71 and 90.0 mg Saytex 102E. The nominal concentration of the predominant PBDE congeners spiked into sand and dredge are listed in Table 1.

Sediment Spiking Procedure

The sediment preparation occurred in four steps after the sediment had been thoroughly dried in a convection oven. There was a total of 6 to 10 days from the time of initial spiking to the first and last set of eggs was incubated. The first step was putting eleven full 250 ml beakers of sand (2 cans) or dredge sediment (2 cans) in four one-gallon Teflon coated aluminum paint cans then adding one liter of hexane and 500 ml of acetone to each of the four paint cans.

These cans were then placed on a homemade mixing apparatus that allowed for constant tumbling of sediment and solvent for 45 minutes each. The second step was the addition of the spiking solution to one can each of sand and dredge. All four cans were tumbled for another 45 minutes. The third step was allowing the solvent to evaporate from the four paint cans in a fume hood. This was done over six days where the cans were capped and shook four times a day. The evaporation was measured by mass loss and by the sixth day evaporation had slowed to where another method to rid the sediment of the remaining solvent was needed. Step four was evaporation over one night in respective glass Pyrex dishes in the fume hood. Before first use of sediments for incubation there was 0.2, 0.2, 0.8 and 1.0% solvent left in sand, dredge, spiked sand and spiked dredge respectively. All cans were tumbled 45 minutes prior to placement in baking dishes. All sediment was then covered with aluminum foil for the remainder of the sediment /incubation preparation.

Incubation

Eggs were incubated singularly in 250 ml clean, hexane rinsed glass beakers. The egg was placed on top of sediment filled to the 100 ml mark then covered with sediment up to the 200 ml mark. The sediment was gently packed to make sure there were no empty pockets. The mass of the egg and sediment were recorded. The beakers were loosely covered with aluminum foil then placed in a Precision Low Temperature Incubator (model #815) set at 28°C. Temperature was chosen based on a study that reported 100% viability and hatchling success

for terrapins incubated artificially at this temperature (Giambanco, 2002). All sediments were gravimetrically prepared to have 4% moisture before incubation began and were checked throughout incubation and water was replaced gravimetrically as needed. The beakers were rotated to different shelves everyday of incubation and the temperature was monitored. At 50 days of incubation, about seven days before estimated hatching, the beakers were removed and the eggs were opened (Giambanco, 2002). Eggs were opened and contents were placed in hexane rinsed glass jars with Teflon lined lids. Live embryo/hatchlings were sacrificed by decapitation. All procedures were approved by a Drexel University Institutional Animal Care and Use Committee protocol (15968). Pictures with measurement scales were taken of each egg and its contents then all contents were frozen in their respective jars at -80 ^{o}C.

Sample Analysis

The calibration solutions used in this study are described in Chapter 2. A new internal standard was used for eggs and sediments that contained ^{13}C-labeled PCB congeners (28, 52, 77, 126, 169, 118, 153, 180, 194 and 206), F-BDE47, BDE 104, F-BDE 160, 4'-F-PBDE 208, ^{13}C labeled PBDE 209, ^{13}C labeled (HCB, *trans*-chlordane, *trans*-nonachlor, oxychlordane, dieldrin, 4,4'-DDE, 4,4'-DDT), 4,4'-DDD-d_8, ^{13}C-labeled alpha, beta, gamma HBCDs and ^{13}C-labeled methyl-triclosan. The sediment internal standard solution also had more ^{13}C labeled PBDE 209 added.

Two NIST SRMs were used as control samples. Three replicates of SRM 1947 (Lake Michigan Fish Tissue) were analyzed with the egg samples in addition to one replicate of an in-house control material of pooled loggerhead eggs (Cc comp). Three replicates of NIST SRM 1941b (Organics in Marine Sediment) were analyzed with the sediment samples. Laboratory procedural blanks were also analyzed.

The egg contents that had formed embryos were homogenized using a tissue homogenizer (Tissue Blender PRO 250). Homogenized and minced egg samples were mixed with diatomaceous earth, spiked with an *iso*-octane internal standard solution, and extracted with dichloromethane (DCM) using pressurized fluid extraction (Dionex Corporation, Sunnyvale, CA) according to (Keller et al., 2004a). These extracts were cleaned up using size exclusion chromatography as described in chapter 3 and also with acidified silica and alumina columns.

Sub samples of pre and post-incubation spiked and non-spiked sediments as well as the 10 nesting beach sediments were analyzed for organic contaminants. Sediment samples were mixed with diatomaceous earth, spiked with an *iso*-octane internal standard solution, and extracted with DCM using pressurized fluid extraction (Dionex Corporation, Sunnyvale, CA) according to Keller et al. (2004a). These extracts were cleaned up using a 7.5 mm x 300 mm (10 µm diameter, 100 Å pore size) PLGel column (Polymer Labs, Amherst, CA) and also with acidified silica columns.

Lipid content was determined gravimetrically from egg initial extracts prior to any clean-up according to (Keller et al., 2004a). Moisture content was determined for all sediments gravimetrically prior to extraction. A subsample of around five grams of sediment was placed in a tared aluminum weighing pan and mass was recorded. Sediments were then placed in a convection oven at 120°C then the mass was recorded once pans reached room temperature under fume hood at 24 and 48 hours.

Final extracts (≈ 0.2 mL in volume) were injected twice on a gas chromatograph mass spectrometer (GC/MS; Agilent Technologies 6890N/5973 inert, Palo Alto, CA) and the limit of detection (LOD) was determined as described in Chapter 2.

Calculations and Statistics

All values that were below the limit of detection (LOD) were converted to half the LOD for the use of statistics and descriptive statistics, but zeros were used when summing contaminant classes. The statistical program SAS version 8.1 (SAS Institute Inc., Cary, NC) was used to run One Way ANOVAs and Student-Newman-Keuls Test for treatment comparisons.

Percent transfer of PBDEs, PCBs and OCPs was calculated using mass balance and control for certain factors when able (in spiked sediments only). For example, the spiked sand percent transfer was calculated by calculating the total nanograms of a compound in the egg contents post incubation and the beakers contents pre-incubation (sediment), then dividing the nanograms in the egg by the

nanograms in the sediment and multiplying by 100. To control for the background contamination in the sediments, the nanograms in the non-spiked sand treatment were subtracted from the spiked sand. To control for background contamination in the egg from maternal transfer and changes to concentrations due to metabolism in the developing embryo we subtracted the nanograms of a compound in the egg incubated in sand from the egg incubated in spiked sand.

SPIKED

$$\left(\frac{\text{(ng in spiked, developed egg - ng in non-spiked, developed egg)}}{\text{(ng in spiked sediment – ng in non-spiked sediment)}} \right) \text{ X } 100$$

= % transfer of compounds from incubation sediments to egg

NON-SPIKED

$$\left(\frac{\text{(ng in non-spiked, developed egg - ng in control, non-incubated egg)}}{\text{ng in non-spiked sediment}} \right) \text{ X } 100$$

= % transfer of compounds from incubation sediments to egg

Results and Discussion

Concentrations measured in all control materials were <11% different from certified values or past mean values for the majority of the compounds. The average differences for all reported values were -10.3 %, 4.7 %, and 4.1% for SRM 1947, Cc Comp and SRM 1941b, respectively. Precision was assessed by comparing the relative standard deviations of three replicates each of SRM 1947 and 1941b which were, on average across all compounds, 2.5% and 14.2%, respectively.

Incubation Sediment Treatments

The spiked sand for incubation was spiked with 11.5 mg of DE-71 (penta-mixture) and 92.6 mg of Saytex 102E (deca-mixture) which should have given the sediment an overall total PBDE concentration of 22.2 µg/g (Table 1). The actual measured mean total PBDE concentration for spiked sand was 13.4 ug/g with 11.5 µg/g being PBDE 209 (Table 1). The spiked dredge for incubation was spiked with 11.6 mg DE-71 and 90.0 mg Saytex 102E that should have given the sediment an overall total PBDE concentration of 24.4 µg/g (Table 1). The actual measured total PBDE concentration for spiked dredge was 14.6 µg/g with 12.8 µg/g being PBDE 209 (Table 1). The non-spiked sand and dredge incubation sediments had average total PBDE concentrations of 0.475 and 0.378 ng/g dry mass, respectively, with PBDE 209 detected in one of nine sand samples at 0.631 ng/g dry mass and none in dredge sediment (Table 1). Overall PBDE concentrations measured in spiked sediments ranged between 11-71% (spiked

sand) and 0-65% (spiked dredge) less than expected based on the amounts of PBDE spiking solution mixed into the sediments. This is most likely due to the volatilization of PBDEs that occurred in the solvent evaporation process when sediments were being prepared. The spiked sediments do however have at least 80 to 120 thousand times more total PBDEs than the non-spiked sediments so we expected to still be able to measure transfer rates of less than ten percent.

The spiked sediments, both sand and dredge acquired the same PBDE pattern as the spiking solutions (Figure 1). The patterns in the non-spiked sediments differed from the spiked sediments. There were fewer congeners detected in the non-spiked sediments and the predominant congeners were dissimilar in comparison to spiked sediments (Figure 1). It is important to note that all sediments used for incubation were baked in an oven to remove all moisture before spiking. This altered the original background concentration and patterns of PBDEs prior to spiking. Because of the baking process during sediment preparation, un-manipulated (pre-baking) sediments were also analyzed for PBDE concentrations. Table 1 reports the pre-baking PBDE concentrations and in comparison to the non-spiked treatments the pre-baking concentrations are greater. We also analyzed natural sediments from nesting beaches throughout Barnegat Bay and report greater concentrations in nesting beach sediment, 2.66 ng/g dry mass total PBDEs, in comparison to non-spiked treatments (Figure 2). It is apparent that the non-spiked treatment sediments underestimate the actual concentrations of PBDEs available to an egg in the wild (Figure 2).

In regards to the known low vapor pressures of PBDEs which allow them to volatilize and travel in air we had to acknowledge that property in our experimental design. We therefore analyzed incubation sediments pre-incubation and post-incubation (Table 1). In some cases there were greater PBDE concentrations in treatment sediments post-incubation which indicated cross contamination during incubation (Table 1). This may be considered a confounding variable that would underestimate the percent transfer calculated in this experiment, however we do not suspect that it had a great effect. In other cases there was a decrease in PBDE concentration post incubation (Table 1). We expected to see a decrease attributable to either uptake of PBDEs by the egg or more likely volatilization. The differences between pre and post concentrations were relatively small suggesting there was little volatilization during the 50 day incubation period (Table 1). When we examine the pre-incubation sediments more closely we can gather more data about the volatility of PBDEs. The incubation sediment preparation procedure continued over ten days (day zero being when PBDEs added to sediment and day 10 when last group of eggs where placed into sediments) and eggs were placed in their respective sediments during three separate days of that 10 day period, therefore we collected pre-incubation sediments that represent each of the three days eggs were placed into sediment (day six, nine and ten). There was large variability between sediment PBDE concentrations on day six and day ten which appears to be due to dry sediments sitting underneath a fume hood (Appendix 1). The longer the sediments sat in the

hood the lower the concentrations became. The rate of loss of PBDEs from sediments was not consistent. For example, the rate of loss from day six to day nine was less that the rate of loss measured between days nine and ten (Appendix 1). Volatilization or loss of PBDEs from incubation sediments during that ten day period was much greater, 50-98% than the 50 day incubation period, under 5% (Appendix 1 and Table 1). It appears that moist sediment retains PBDEs better at warmer temperatures over a longer period of time than dry sediment at cooler temperatures over a shorter period of time.

What we can suggest in future experiments that spiked sediments should be placed in separate incubators that are equipped with air scrubbers so the air coming out of the incubator does not contaminate other incubators in the room. We can also suggest that sediment preparation time and procedures be taken into account when using PBDEs. If PBDEs are left in dry sediment, even at cooler temperatures there can be a relatively rapid loss of the compounds. A better containment method for spiked sediments should be considered to decrease loss of compounds and contamination of other materials.

Egg Incubation

Out of the 40 eggs incubated, 20 were incubated in sand treatments and 20 were incubated in dredge treatments. There was no development in any dredged sediments, non-spiked or spiked. This was due to the negative water potential of the dredged sediment determined after the experiment. All eggs were desiccated in the dredge sediment (Figure 3E). Even though the net movement of water was

from the egg into the incubating sediment there was still a transfer of PBDEs into the egg contents (Table 2). The sand and spiked sand sediments yielded 15 fully formed live embryo/hatchlings, 2 partially formed embryos and 3 embryos that showed signs of early development (Table 3, Figure 3A-D). We expected to see fully formed embryos/hatchlings because incubation was stopped about seven days before the predicted hatch date, based on temperature of incubation, date of ovoposition and start of incubation. Although incubation in both sand and spiked sand produced a similar number of fully formed individuals there appeared to be more occurrences of scute anomalies in the spiked sand embryos, where seven of the 10 had an anomaly of some kind (Table 3). Anomalies were recorded and the occurrence of a split nuchal scute or the odd number and pattern or placement of scutes on the carapace (examples: Figure 3A&B).

The ΣPBDE concentrations in the eggs incubated in spiked treatments (both sand and dredge) were significantly greater than the eggs incubated in non-spiked treatments indicating PBDEs did transfer from sediment into the eggs (Table 2). The eggs incubated in the spiked sand, those that actually developed, showed significantly greater ΣPBDE concentrations than the eggs that did not develop in the spiked dredge indicating greater transfer of PBDEs with live developing embryos (Table 2). No significant differences were observed in ΣPBDE concentration between the eggs incubated in the non-spiked treatments (both sand and dredge) and the control eggs (Table 2).

Percent Transfer

The percent transfer reported in this study represents the uptake and transfer of compounds from incubation sediments into the egg contents. Because all egg contents were homogenized (minus the egg shell) for contaminant analysis we cannot predict if the compounds were partitioned in the embryo itself or the membranes and other contents. The reported values will predict the amount of compounds that are available to the embryo because it has passed through the egg shell. Equally important, the amount of sediment that touched the eggs or was capable of releasing contaminants for transfer into the egg is unknown, but is likely smaller than the total mass of sediment in each beaker. For this reason and because the mass of contaminants in the sediment of the entire beaker was used as the denominator, the percent transfer is likely underestimated and would always be dependent upon the mass of sediment used in the experiment. This is an important consideration if the current results are ever compared to another transfer study.

The compounds listed in Table 4 are those that showed detectable transfer into the eggs from treatment sediments. Lower brominated PBDEs like 47 and 66 consistently showed the greatest percent transfer in the spiked sediments (Table 4). Spiked sand percent transfer ranged from 0.0001% (PBDE 209) to 0.7883% (PBDE 47) and spiked dredge ranged from 0.0003% (PBDE 209) to 1.58% (PBDE 66) (Table 4). It was not surprising that the lower brominated compounds have the highest percent transfer, because PBDE 47 has been reported to be the

most bioavailable congener where as PBDE 209 is a very large and bulky and does not travel through biological barriers as easily (de Wit, 2002). In the non-spiked sediments percent transfer for only one PBDE congener, PBDE 154, could be calculated (Table 4). This was because there were no other congeners detected in both the non-spiked sediments and the eggs (Table 1 and 2). The percent transfer for PBDE 154 was much greater from the non-spiked sediments in comparison to the spiked sediments, suggesting that either transfer is concentration dependent, the adsorption of PBDE 154 to non-spiked sediments differs from that to the spiked treatments, or biological processes differed between the spiked vs. non-spiked to result in greater transfer in the non-spiked. This congener also happens to be one of the most predominant congeners found in Barnegat Bay terrapin tissues and eggs (Chapters 2 and 3).

One of our major goals of this study was to determine the difference in transfer of PBDEs from sand nesting sediments and the dredged sediments. Unfortunately, due to the lack of development in the dredged sediment treatments we are not able to make any hypotheses as to which sediment type facilitates the greatest transfer. We can however make a hypothesis that developing eggs (those is sand treatments) facilitate a higher percent transfer for the majority of the compounds in Table 4 compared to non-developing eggs (those in dredge treatments). The greater transfer in developing eggs can be attributed to the ability of PBDEs and other POPs to pass through the shell during gas exchange. Because PBDEs, like other organic contaminants volatilize, they become

available in vapor form to diffuse through the shell and pores similar to the oxygen and carbon dioxide being exchanged during development. This hypothesis was also suggested in a study where organochlorine pesticide (OCP) transfer was measured from mixed natural and artificial nesting material to bullsnake (*Pituophis melanoleucus*) eggs (Canas and Anderson, 2002). We can also compare the ability of PBDEs and other POPs to transfer from sand into developing eggs to other uptake and transfer studies. Additionally, the lack of development in the dredge sediment still allowed transfer, suggesting that there are passive transfer mechanisms in addition to active ones that were at work in the developing sand-incubated eggs. This proposed hypothesis (higher transfer from more biological activity) is confounded by the differences in organic matter and clay content between the two sediment types. Both sediment characteristics and biological activity differed between the sand and dredge treatments, so both could contribute to the differences seen in percent transfer.

Although other POPs and OCPs were not the main focus of this study we were able measure % transfer of the compounds listed in Table 4. These were compounds that were detectable in the majority of sediments samples. The two PCB congeners 180+193 and 206 had greater transfer in sand treatments where there were developing embryos (Table 4). The lower chlorinated congeners had greater percent transfer; similar to what was observed in the spiked PBDE treatments. The PCB congeners (180+193) with the greatest transfer also happen to be a predominant congener in terrapin tissues (Chapters 2 and 3). Percent

transfer for mirex and DDT metabolites were also reported in Table 4. Mirex unexpectedly had greater transfer in dredge sediment whereas the most environmentally relevant DDT metabolite in animal tissues, 4,4'-DDE, had greater transfer in the sand treatment where there were developing embryos (Table 4). It is possible that the mirex transferred through the egg shell during development was chemically modified by the developing embryo therefore making the compounds "disappear" from the analysis of the egg which in turn produced a lower percent transfer than what was seen in sediment with no developing embryos. The percent transfer calculation from spiked sediments should have controlled for the effects of biotransformation as well as background contamination from maternal transfer by subtracting the concentrations from the non-spiked treatment eggs, where as in the non-spiked treatments we were not able to subtract the effect of biotransformation, only background contamination from maternal transfer through the non-incubated control egg.

Many studies of POP transfer/uptake through egg shells or into developing embryos is conducted by either topical application of compounds directly to egg shell or injecting the compounds into egg air cells. For example, 34% of 4,4'-DDE painted onto a green sea turtle (*Chelonia mydas*) egg was absorbed into the egg but only 8% made it into the embryo (Podreka et al., 1998). In another study 90% and 96% of PCB 126 and TCDD respectively passed through the red eared slider turtle shells after topical application but only 1.7% and 0.8% of original dose made it into the embryo (Gale et al., 2002). In a study where a penta-PBDE

mixture was injected into the air cells of American Kestrel (*Falco sparverius*) and chicken (*Gallus gallus*) eggs, 18% and 29% of the original dose was represented in the egg contents respectively (McKernan et al., 2009). These three studies deal with direct application of the contaminant which may elicit a toxic effect that they are looking to quantify but it does not represent the true potential for the uptake of contaminants in the wild. To date this is the only study to measure transfer of PBDEs and other POPs from completely natural nesting sediments into reptile eggs. There is a study where the nest material, a mixture of Spanish moss and vermiculite facilitated transfer ranging from 0.5% (Dieldrin) to 19% (Lindane) with no detectable transfer of 4,4'-DDE into bullsnake eggs (Canas and Anderson, 2002). This study does suggest there is potential for OCPs to transfer from nest material but it is still not completely natural and this study does not include PCBs and PBDEs. The current study, although it has much lower percentages of transfer, shows what may occur in the natural nesting environment of the terrapin and that PBDEs and other POPs will transfer through the eggshell into egg contents.

Consequences of Contaminant Transfer to Developing Terrapins

We did not find any obvious delays in development in the embryos incubated in the spiked sand compared to the non-spiked sand. The occurrence of partially formed and early development embryos seemed to be more related to the clutch the eggs came from rather than the treatment they were incubated in. We did notice a higher occurrence of carapace vertebral and marginal scute anomalies

in the spiked sand treatments, which on average had concentrations about 150 times greater than the maximum control egg, when compared to the sand treatments (Table 3, Figure 3A and 3B). There were no correlations between the ΣPBDE concentrations and carapace length or final egg mass (post incubation but before opening). There were also no significant differences in egg or embryo mass, or carapace and plastron length between those incubated in sand versus the spiked sand (Figure 4). Although there were no outward signs of delays in development or deformities we cannot disregard the internal effects that may have occurred due to the high PBDE concentrations in the spiked sand embryos. PBDEs have been reported to cause endocrine disruption and developmental neurotoxicity including learning and memory into adulthood (Eriksson et al., 1999; Timme-Laragy et al., 2006; Darnerud, 2008). These internal effects are often more sensitive than external effects like deformities, and neurobehavioral effects have been reported to be the most sensitive endpoint in regard to PBDE toxicity (Chapter 5). In killifish (*Fundulus heteroclitus*) embryos exposed to DE-71, 0-7 days post fertilization, experienced hypoactivity and the elimination of normal fright response causing higher predation rates low doses, in the 0.001 and 0.01 ug/L exposure groups (Timme-Laragy et al., 2006). In the current study, terrapin PBDE concentrations incubated in spiked sand (96.5- 318 ng/g) and sand (0.256 – 3.14 ng/g) are within and above the range of concentrations that caused the neurobehavioral effects in killifish (Table 3).

It is important to note that as discussed above, the levels of PBDEs in the non-spiked incubation treatments were underestimated due to three factors; (1) the assumptions of the mass balanced percent transfer calculations, (2) cross contamination of PBDEs during incubations and (3) to a baking step in the sediment preparation process (Figure 2). In comparison to pre-baked and natural nesting beach sediments, eggs incubating in a natural environment may be exposed to greater PBDE concentrations thereby elevating the actual level of PBDEs available to the developing embryo. By taking the percent transfer calculated through the transfer study we can estimate how much contamination that an egg incubating in nesting beach sediment may accumulate. PBDE 154 is the only congener that is detected in both the nesting beach sediments (0.186 ng/g dry mass) and the control eggs (0.104 ng/g wet mass). If there is a 1.67 percent transfer of PBDE 154 from non-spiked sand to the egg we may expect an additional 0.03 ng/g of PBDE 154 to be available to the incubating egg. This is obviously a small amount, an amount that may not be large enough to elicit a toxic effect based on the amount transferred alone. Although we did measure transfer of PBDEs in incubation sediments, the different PBDE profiles seen in non-spiked sediments in comparison to eggs incubated in those sediments, and low PBDE concentrations indicates that PBDE transfer from nesting sediments can be negligible compared to the major route of exposure to a developing embryo which would be maternal transfer. The data reported in this study suggest that terrapins and other land nesting species in Barnegat Bay may not accumulate

a substantial amount of PBDEs or other POPs through sandy nesting sediments but terrapins in other more contaminated areas may be at risk.

Table 1. Summary of PBDE concentrations in incubation sediments both before and after incubation as well as the estimated concentration of the spiked sediments based on the composition spiking solution prepared for incubation sediments and the actual mass of spiking solution that was added to sediments. For congeners that have no samples detected above the limit of detection the descriptive statistics represent the mean, standard deviation and range of half the limit of detection for that compound. Concentrations for pre-baking sediments are also included as well as the calculated % difference between the pre-incubation sediments to post-incubation sediments.

	SPIKING SOLUTION			PRE-INCUBATION SEDIMENT											
				Spiked Sand (ng/g) n=9			Spiked Dredge (ng/g) n=3			Sand (ng/g) n=9			Dredge (ng/g) n=3		
	% Congner in Spiking Solution	Expected Conc. in Spiked Sand (ng/g)	Expected Conc. in Spiked Dredge (ng/g)	Mean (stdev)	Range	n>LOD	Mean (stdev)	Range	n>LOD	Mean (stdev)	Range	n>LOD	Mean (stdev)	Range	n>LOD
47	3.72	826	906	456 (189)	186 - 664	9	490 (362)	238 - 905	3	0.043 (0.002)	0.038 - 0.046	0	0.044 (0.001)	0.044 -0.045	0
66	0.04	9.78	10.7	3.50 (1.82)	1.51 -5.67	9	3.24 (1.76)	1.97 - 5.25	3	0.012 (0.001)	0.009 - 0.013	0	0.012 (0.001)	0.012 - 0.013	0
85*	0.35	77.54	85.04	19.1 (10.9)	6.57 - 33.3	9	17 (9.92)	9.57 - 28.3	3	0.155 (0.021)	0.102 - 0.168	0	0.163 (0.004)	0.160 -0.167	0
99	4.71	1046	1148	594 (292)	215 - 919	9	638 (531)	238 - 1248	3	0.092 (0.015)	0.052 - 0.101	0	0.097 (0.002)	0.096 - 0.100	0
100	1.05	233	256	214 (79.2)	93.9 - 294	9	223 (136)	120 - 377	3	0.186 (0.078)	0.093 - 0.330	8	0.220 (0.068)	0.159 - 0.293	1
138	0.102	22.7	24.9	2.75 (1.57)	1.02 - 4.83	9	2.15 (0.946)	1.42 - 3.22	3	0.117 (0.009)	0.097 - 0.128	9	0.108 (0.016)	0.090 - 0.120	3
153	0.355	78.9	86.5	26.8 (13.1)	10.7 - 42.6	9	23.9 (14.0)	15.1 - 40.0	3	0.035 (0.003)	0.026 - 0.037	0	0.036 (0.001)	0.035 - 0.037	0
154	0.274	60.9	66.8	27.9 (13.3)	10.9 - 42.6	9	25.2 (13.7)	15.5 - 40.8	3	0.106 (0.050)	0.068 - 0.194	3	0.097 (0.043)	0.070 - 0.147	1
156	0	0	0	0.206 (0.178)	0.020 - 0.410	2	0.152 (0.230)	0.017 - 0.417		0.137 (0.324)	0.052 - 0.154	1	0.149 (0.003)	0.147 - 0.153	0
181	0.076	16.9	18.5	0.206 (0.262)	0.019 - 0.840	3	0.126 (0.011)	0.118 - 0.139	2	0.049 (0.003)	0.043 - 0.051	1	0.050 (0.001)	0.049 - 0.051	0
209	85.8	19072	20917	11501 (4924)	5386 - 17197	9	12847 (7817)	7437 - 21810	3	0.350 (0.106)	0.289 - 0.631	1	0.314 (0.007)	0.309 - 0.322	0
ΣPBDEs	100	22218	24367	13373 (5564)	6461 - 19820	9	14639 (8984)	8436 - 24942	3	0.475 (0.263)	0.209 - 0.931	9	0.378 (0.158)	0.274 - 0.560	3
				POST INCUBATION SEDIMENT											
				n=3			n=3			n=3			n=3		
47	–	–	–	434 (226)	175 - 595	3	541 (291)	346 -875	3	0.142 (0.169)	0.041 - 0.337	1	0.042 (0.001)	0.041 - 0.043	0
66	–	–	–	3.43 (2.13)	1.02 - 5.07	3	4.26 (2.49)	2.05 - 6.96	3	1.02 (1.74)	0.011 - 3.02	1	0.012 (0.001)	0.011 - 0.012	0
85*	–	–	–	20.4 (12.6)	6.07 - 29.6	3	21.3 (14.3)	11.1 - 37.7	3	0.152 (0.029)	0.124 - 0.182	0	0.154 (0.003)	0.151 - 0.157	0
99	–	–	–	585 (324)	213 - 810	3	712 (454)	435 - 1235	3	0.127 (0.088)	0.064 - 0.227	1	0.092 (0.002)	0.090 - 0.094	0
100	–	–	–	194 (92.4)	87.9 - 256	3	261 (122)	171 - 400	3	0.157 (0.038)	0.114 - 0.182	2	0.188 (0.057)	0.133 - 0.248	3
138	–	–	–	2.55 (1.35)	1.037 - 3.65	3	2.91 (2.06)	1.50 - 5.27	3	0.135 (0.045)	0.0945 - 0.183	3	0.101 (0.015)	0.089 - 0.117	3
153	–	–	–	25.7 (13.0)	10.9 - 35.2	3	30.2 (18.6)	19.3 - 51.6	3	0.035 (0.004)	0.032 - 0.040	0	0.034 (0.001)	0.033 - 0.035	3
154	–	–	–	27.2 (13.8)	11.3 - 36.4	3	32.4 (19.1)	20.0 - 51.6	3	0.274 (0.316)	0.080 - 0.639	1	0.096 (0.049)	0.066 - 0.153	1
156	–	–	–	0.273 (0.220)	0.018 - 0.407	0	0.131 (0.193)	0.019 - 0.355	0	0.109 (0.076)	0.023 - 0.166	0	0.141 (0.003)	0.138 - 0.144	3
181	–	–	–	0.095 (0.067)	0.017 - 0.136	0	0.190 (0.080)	0.118 - 0.276	2	0.052 (0.005)	0.046 - 0.055	1	0.047 (0.001)	0.046 - 0.048	3
209	–	–	–	11600 (5530)	5190 - 15200	3	14500 (7890)	8510 - 23500	3	0.347 (0.055)	0.291 - 0.400	0	0.297 (0.007)	0.291 - 0.304	0
ΣPBDEs	–	–	–	13628 (5950)	6810 - 17800	3	16500 (8900)	9930 - 26700	3	3.22 (4.65)	0.182 - 8.57	3	0.359 (0.096)	0.272 - 0.462	3

Table 1. Continued.

	PRE-BAKING SEDIMENT				% Difference Pre-incubation to Post-Incubation			
	Sand (ng/g) n=3 Mean (stdev)	n>LOD	Dredge (ng/g) n=3 Mean (stdev)	n>LOD	Spiked Sand	Spiked Dredge	Sand	Dredge
47	<0.237	0	<0.083	0	5.28	-10.2		
66	<0.056	0	<0.020	0	1.91	-31.2		
85*	<0.329	0	<0.220	0	-7.00	-24.9		
99	<0.323	0	<0.113	0	1.52	-11.6		
100	<0.576	0	0<0.202	0	9.06	-17.2	32.2	14.8
138	0.322 (0.035)	3	0.109 (0.002)	1	7.17	-35.3	-15.4	6.85
153	<0.162	0	<0.057	0	3.84	-26.4		
154	1.02 (0.600)	3	0.294 (0.350)	2	2.58	-28.6	-275	-3.77
156	0.173 (0.008)	3	0.060 (0.006)	2				
181	0.138 (0.016)	3	0.050 (0.004)	3		-87.9	-279	
209	<0.690	0	0.671 (0.575)	2	-0.46	-13.1		
ΣPBDEs	1.65 (0.564)	3	1.11 (0.233)	3	-1.91	-13.0	-3641	4.97

* includes PBDE 155 because co-elutes on column where it was measured.
<LOD = below the limit of detection
% difference is (-) means more of that congener present in post incubation sediments.

Table 2. Summary of PBDE concentrations in the egg contents from all four incubation treatments as well as the frozen control egg (representing background contamination). The letters in bold next to the ΣPBDE values are showing the treatments that were statistically different from each other.

	Eggs from Spiked Sand n = 9			Eggs from Spiked Dredge n = 7			Eggs from Sand n = 9			Eggs from Dredge n = 7			Control Eggs n =10		
	Mean	Stdev	n>LOD	Mean	Stdev	n>LOD	Mean	Stdev	n>LOD	Mean	Stdev	n>LOD	Mean	Stdev	n>LOD
PBDE 47	117	56.1	9	10.2	4.08	7	0.303	0.516	9	0.195	0.126	7	0.103	0.124	10
PBDE 66	0.534	0.331	8	1.38	3.51	7	0.003	0.002	1	0.006	0.006	1	0.003	0.002	4
PBDE 85	1.25	0.560	9	0.192	0.088	6	0.076	0.057	0	0.124	0.109	0	0.046	0.008	0
PBDE 99	35.5	15.1	9	3.03	1.34	7	0.135	0.157	9	0.175	0.156	7	0.072	0.040	10
PBDE 100	14.0	5.89	9	0.991	0.644	7	0.006	0.004	0	0.009	0.008	0	0.003	0.001	0
PBDE 153	0.634	0.220	9	0.31	0.195	7	0.169	0.101	9	0.314	0.255	7	0.123	0.073	10
PBDE 154	0.777	0.429	9	0.143	0.061	7	0.167	0.132	8	0.267	0.190	7	0.104	0.027	10
PBDE 209	1.78	3.35	7	4.76	2.88	7	0.169	0.113	1	0.244	0.215	0	0.091	0.016	0
∑PBDEs	175 **A**	78.7	9	22.3 **B**	7.56	7	1.17 **C**	0.943	9	1.46 **C**	1.02	7	0.608 **C**	0.253	10

<LOD = below limit of detection

Table 3. Summary of egg content/ embryo descriptions in all four incubation treatments (n=10 eggs per treatment; one egg from 10 clutches).

	Spiked Sand	Sand	Spiked Dredge	Dredge
Fully Formed	7	8	0	0
Partially Formed[a]	2	1	0	0
Signs of early development	1	1	0	0
No development	0	0	10	10
Scute Anomalies[b]	7	3	0	0

[a] Partially formed include embryos with arrested development and have at least head development with eyespots.
[b] Scute anomalies were assessed in only the fully formed embryos and include occurrence of split nuchal, odd number and/or pattern of scutes.

Table 4. Mean percent transfer of total PBDEs from incubating sediments into eggs. The sample sizes listed in the table are the number of samples analyzed in each treatment. Spiked sand and sand percent transfer represent transfer into egg with developing embryos. Compounds with no reported value are those that were not detected in both the sediment and egg of that treatment.

	Spiked Sand, n=9		Spiked Dredge, n=3		Sand, n=9		Dredge, n=3	
	Avg	Stdev	Avg	Stdev	Avg	Stdev	Avg	Stdev
PBDE 47	0.7883	0.3941	0.0240	0.0181	–	–	–	–
PBDE 66	0.4605	0.3587	1.5783	2.7151	–	–	–	–
PBDE 100	0.1978	0.0883	0.0048	0.0043	–	–	–	–
PBDE 99	0.2052	0.1185	0.0063	0.0051	–	–	–	–
PBDE 85+155	0.2341	0.1426	0.0016	0.0065	–	–	–	–
PBDE 154	0.0765	0.0528	0.0063	0.0136	1.666	7.033	1.078	1.047
PBDE 153	0.0617	0.0377	0.0014	0.0043	–	–	–	–
PBDE 206	0.0001	0.0013	–	–	–	–	–	–
PBDE 209	0.0006	0.0014	0.0003	0.0003	–	–	–	–
PCB 180+193	–	–	0.4774	1.4748	2.223	7.959	0.3015	0.2566
PCB 206	0.4944	2.5269	–	–	0.4467	1.490	0.0863	0.0840
Mirex			–	–	0.1182	0.2512	0.5662	0.5444
2,4'-DDE	–	–	0.4810	0.8386	0.0054	0.0473	0.0569	0.0976
4,4'-DDE	–	–	–	–	0.9297	7.182	0.2806	0.3204
2,4'-DDD	–	–	–	–	0.0138	0.0414	0.6638	1.150
4,4'-DDD	0.0154	0.0434	0.0661	0.0865	0.0051	0.0237	0.0053	0.0039
2,4'-DDT	0.0023	0.0209	0.0019	0.0427	0.0282	0.0847	0.0192	0.0182
4,4'-DDT	–	–	0.0045	0.0546	0.0609	0.1872	0.0009	0.0072

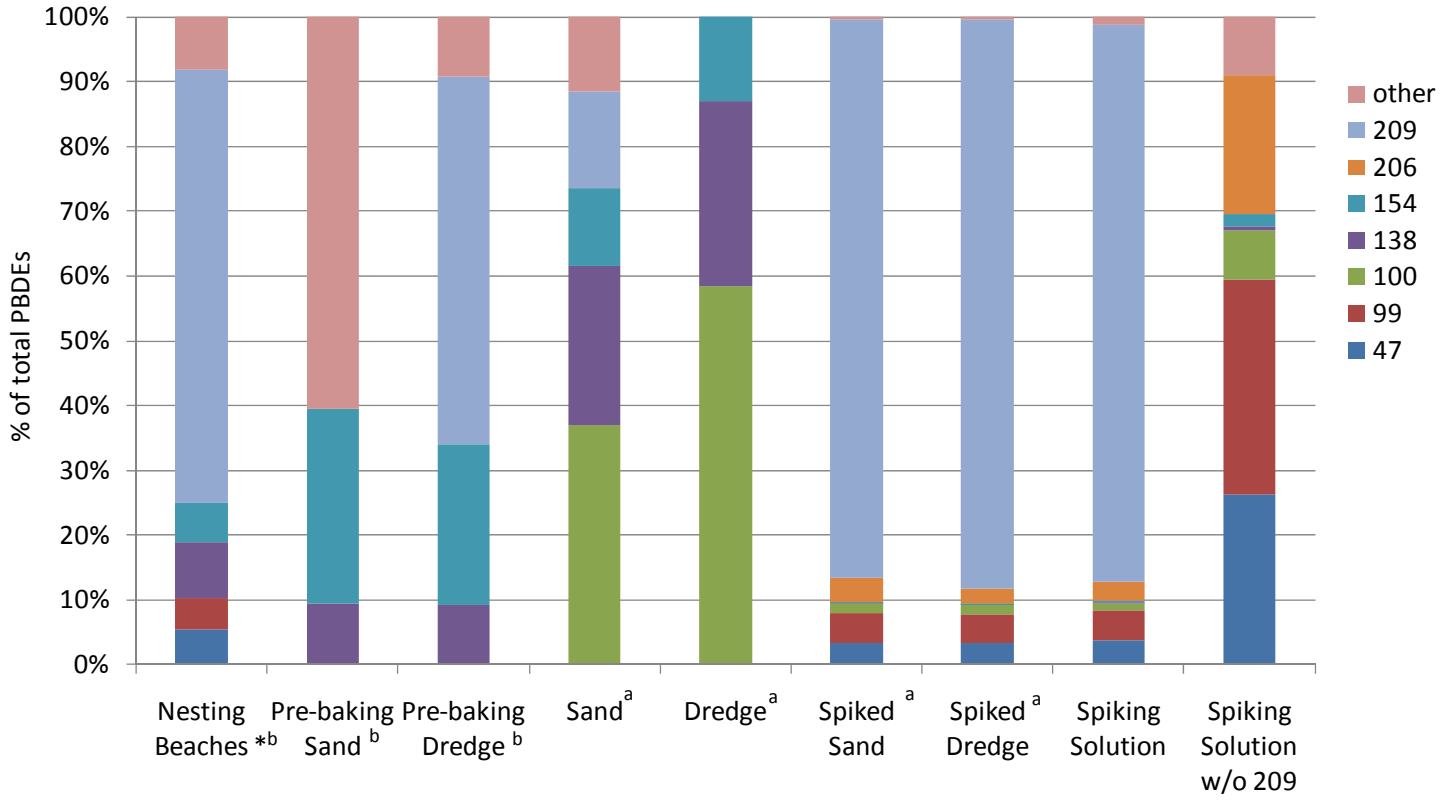

Figure 1. Mean PBDE congener composition in treatment sediments compared to natural un-manipulated sediments and the spiking solutions used to spike the sediments. Percents represent portion of the sum of all PBDE congeners detectable. Other refers to those that were detectable but not specified in figure.

* PBDE 47 and 99 only detected in one of ten beaches. This bar is an average of all ten nesting beach sediment samples.

[a] Sediments manipulated and used as incubation treatments.

[b] Natural un-manipulated sediments.
"other" = non-predominant congeners included in sum.
Spiked sediments: PBDEs 17,25,33+28, 75, 49+71, 66, 119, 85+155, 153, 183, 191, 190, 203, (156, 181 –Spiked sand only).
Sand: PBDEs 17, 156, 181
Nesting Beaches and Pre-baked sediments: PBDEs 156, 181

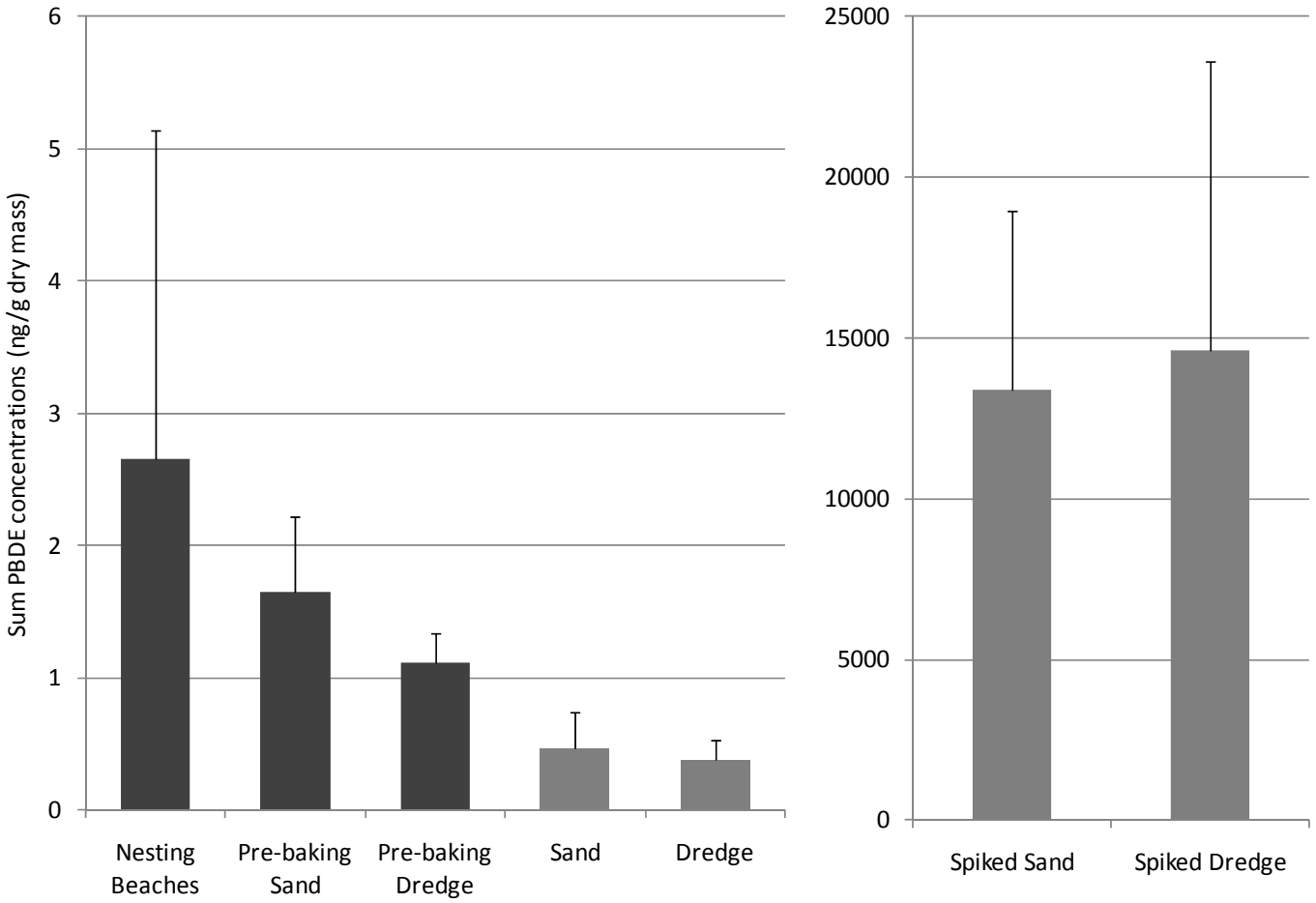

Figure 2. Concentrations of the ΣPBDEs in the natural un-manipulated sediments (black bars) and the treatment sediments (gray bars). Error bars represent standard deviation.

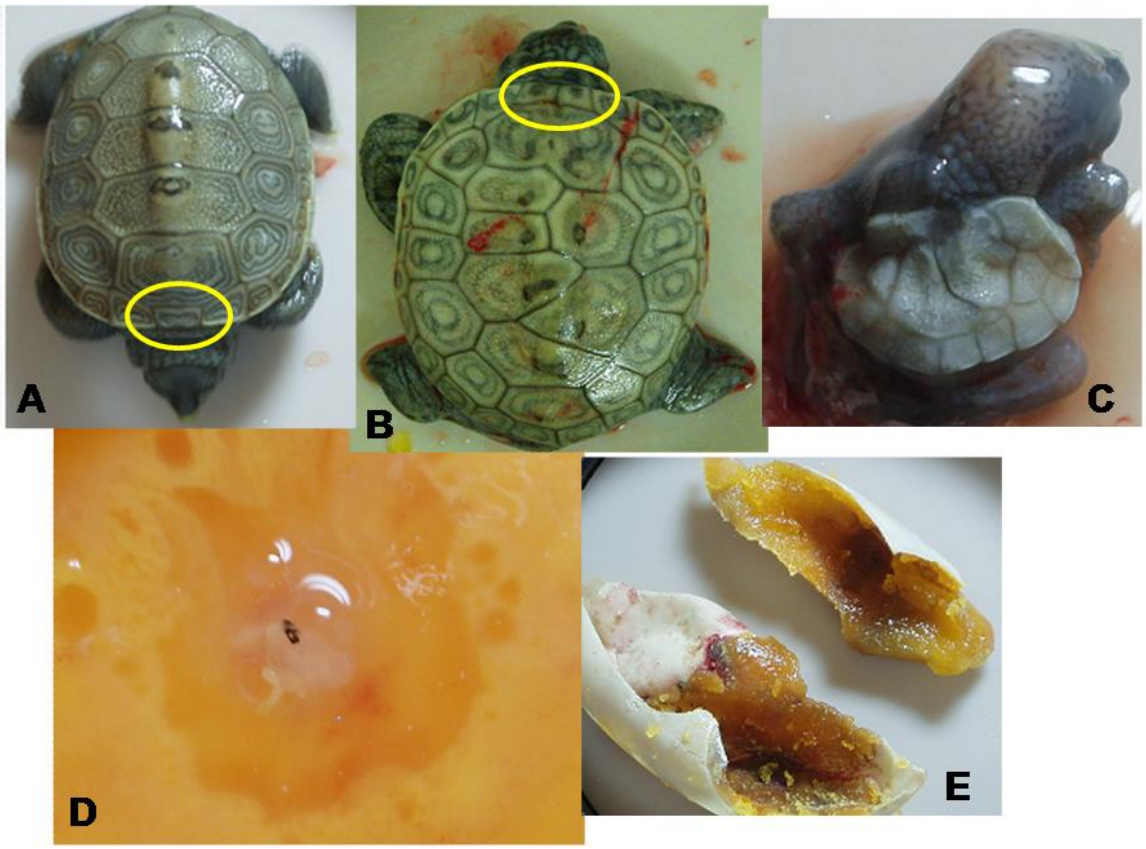

Figure 3. Pictures of egg contents from incubation study. A.) Fully formed embryo/ hatchling incubated in sand and normal nuchal scute. B.) Fully formed embryo incubated in spiked sand with vertebral scute anomalies and split nuchal. C.) Deformed embryo, incubated in spiked sand. D.) Egg with signs of initial development, incubated in spiked sand. E.) Desiccated egg and its contents, incubated in dredge material.

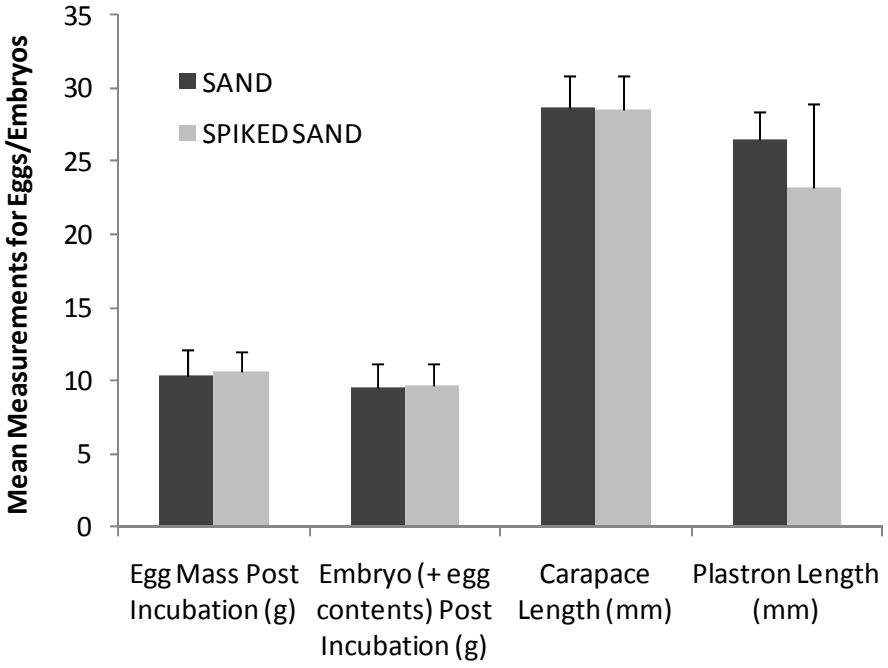

Figure 4. Summary of egg and live embryo morphometrics incubated in the two sand treatments. Error bars represent standard deviation from the mean.

CHAPTER 5:
EXAMINATION OF ENDOCRINE DISRUPTION, IMMUNOTOXICITY AND NEUROTOXICITY BY PERSISTENT ORGANIC POLLUTANTS AT ENVIRONMENTAL CONCENTRATIONS IN DIAMONDBACK TERRAPINS OF BARNEGAT BAY, NJ

Abstract

Persistent organic pollutants (POPs) have been known to cause toxic effects on sensitive physiological systems for decades. In this study 15 adult female terrapin plasma samples, ten non-incubated terrapin eggs and 54 terrapin hatchlings were used to examine the associations between environmentally relevant concentrations of persistent organic pollutants (POPs) and three indicators of health. Significant negative correlations were seen between female terrapin ΣPCB (r_p = -0.6667, p= 0.031), ΣTCDD-like PCBs (r_p = -0.6223, p= 0.031) and ΣPBDE (r_p = -0.6097, p= 0.016) concentrations and total circulating plasma thyroxine (T4) levels suggesting endocrine disruption. Positive significant correlations were seen between plasma lysozyme activity and female terrapin plasma mirex (r_s = 0.6034, p= 0.017), ΣPCB (r_s = 0.5139, p= 0.050), ΣTCDD-like PCBs (r_s = 0.6142, p= 0.015) and ΣPBDE (r_p 0.5603, p= 0.030) concentrations suggesting immunoenhancement. Terrapin egg concentrations of PBDE 47 were significantly correlated with longer righting responses of hatchlings (r_p = 0.5861, p= 0.045) suggesting neurobehavioral toxicity. This data suggests that the terrapin population of Barnegat Bay, NJ may be at risk for sublethal toxic effects that might lead to decreased survival and fitness due to the levels of POPs

accumulated in their tissues. Because we are seeing associations between terrapin POP concentrations and certain health indicators, terrapins may be useful bioindicators for endocrine disruption and immunotoxicity caused by environmental concentrations of POPs in the estuarine environment in respect to other species and humans.

Introduction

Persistent organic pollutants (POPs) are often referred to as ubiquitous. They are found in many compartments of the terrestrial and aquatic environments, including wildlife inhabiting those environments. Because biomagnification and bioaccumulation in fatty tissues are characteristic of POPs, the toxicity of these chemicals in wildlife becomes a concern. Reproductive effects, carcinogenicity, immunotoxicity, neurotoxicity and endocrine disruption are all possible toxic health endpoints to animals and humans exposed to POPs (Eriksson et al., 1999; Jones and de Voogt, 1999; Eriksson et al., 2006). In this study we focused on toxicity endpoints that span endocrine disruption, immunotoxicity and neurotoxicity of POPs in the diamondback terrapin (*Malaclemys terrapin*). The diamondback terrapin is mid-sized, long lived, strictly estuarine reptile that can be found in estuaries along the east coast from Rhode Island to the Gulf of Mexico. Because terrapins are habitat generalists with a long life span, a wide geographic distribution, that exist in a variety of habitats, have a high trophic position and exhibit site fidelity they are excellent species to use as bioindicators (Blanvillain et al., 2007). They have been suggested as good bioindicators of POP

contamination of Barnegat Bay in previous chapters (Chapters 2-4). In this chapter however they will be evaluated as bioindicators of toxic health endpoints associated with their environmentally relevant levels of POP exposure.

Endocrine Disruption

In mammals thyroid hormones are responsible for regulating the metabolic rate of all cells, sexual maturation and temperature regulation. They also regulate cell growth and tissue differentiation including the development of the brain and the central nervous system (Thibodeau and Patton, 2003; Neave, 2008). In amphibians an important function of thyroid hormones is metamorphosis. In reptiles, the thyroid hormone system is responsible for growth in both embryonic and post-embryonic stages, the hatching process and reproduction. It may also aid in metabolism and growth when animals are at or close to preferred temperatures (Hulbert and Williams, 1988). The proper functioning of thyroid hormone homeostasis can be monitored by measuring the levels of thyroid hormones in the plasma of the test species. Disruptions in the levels of total and free circulating hormones can indicate disruption and have previously been linked to concentrations of POPs (Brown et al., 2004). We expect to see significant and negative correlations between POP and thyroxine (T4) concentrations in the terrapins, because POPs, especially polybrominated diphenyl ethers (PBDEs), are known to alter thyroid hormones in mammals, birds and fish (Hallgren and Darnerud, 2002; Brown et al., 2004; Fernie et al., 2005; Kuriyama et al., 2007).

Immunotoxicity

The reptilian immune system is very similar to all vertebrate immune systems. It is capable of fighting bacterial, viral, fungal and parasitic infections as well as fighting tumor cells (Keller et al., 2006b). Reptiles have a more complex and developed immune system in comparison to fish and amphibians and share similarities to higher vertebrates like birds and mammals (Keller et al., 2006b). The reptilian immune system is comprised of the adaptive immune system and the innate immune system which has both cell-mediated and humoral immune responses (Keller et al., 2006b). The innate immune system is often thought to be primitive, but it has evolved alongside adaptive responses, and is actually considered the most important aspect of immunity because without the proper function and occurrence of innate immune cells and responses, the adaptive immune system would not be triggered and would be ineffectual (Beutler, 2004). For the purpose of this study we focused on the innate immune system. We chose to measure lysozyme activity as an indicator of immune function in terrapins, because it has consistently correlated with environmental contaminants (PCBs, mercury) in other reptilian species and because it does not require captivity or lethal sampling (Keller et al., 2004b; Blanvillain, 2005; Keller et al., 2006b; Day et al., 2007). Lysozyme activity is also believed to be extremely important in reptile immunity. Biologically, circulating lysozyme is responsible for pro-inflammatory responses as well as having ant-bacterial properties (Mock and Peters, 1990; Burton et al., 2002). We expect to see decreased lysozyme

activity with increased POP concentrations for two reasons. The first reason is because decreases in lysozyme activity have been reported in correlation to environmental POP and heavy metal concentrations (Keller et al., 2004b; Blanvillain, 2005). The second reason is that immune system has been previously reported to be sensitive to POPs, more sensitive than other physiological responses or biomarkers, so we expect to see a response at the low concentrations in terrapins from Barnegat Bay (Dickerson et al., 1994).

Neurotoxicity

Neurotoxicity can be measured through many different tests and many different stages of development and growth. We measured neurotoxicity, by specifically looking for disruptions in neurobehavioral development. The righting response in turtles is a common behavioral test generally used to measure the effects of different stressors (Steyermark and Spotila, 2001). There is an ecological significance for a hatchling to right itself. If hatchlings are not able to right after emerging from the nest they may become subject to higher rates of predation, desiccation and overheating (Steyermark and Spotila, 2001). Righting response has also been used to measure neurobehavioral responses (Hays, 2005). In this study we are using the response as a tool to compare the environmentally relevant POP concentrations in a clutch to the ability of the hatchling to right itself. We are interpreting a prolonged response as suggestive of a disruption in neurobehavioral development based on data reported in other studies evaluating righting response in relation to contaminants (Burger et al., 1998; Van Meter et

al., 2006). We chose to assess neurobehavioral toxicity in terrapins because neurotoxicity has been reported to be the most sensitive toxic endpoint for PBDEs with lowest observed effects seen at concentrations of 0.001 µg/ l of DE-71 which is a penta-BDE technical mixture (Timme-Laragy et al., 2006). In killifish low doses of DE-71 caused altered activity, fright response and predation rates and learning ability (Timme-Laragy et al., 2006). In mice PBDE 47, 99, 153 and 209 has been reported to cause disruptions in neurobehavioral development such as permanent aberrations in spontaneous behavior and learning and memory functions that worsen with age (Eriksson et al., 1998; Viberg et al., 2003; Johansson et al., 2008).

The main objective of this study was to examine associations between measures of endocrine, immune and neurodevelopmental health at environmentally relevant levels of POPs in diamondback terrapins. Secondarily, the resulting information from these associations may provide information on suitable biomarkers of endocrine and immune disruption by POPs through taking a non-lethal blood sample from a model estuarine vertebrate. This may expand the use of the diamondback terrapin as not only an indicator of POP contamination in Barnegat Bay but also as an indicator of toxic health endpoints associated with those environmental POPs concentrations.

Materials and Methods

Study Site and Sample Collection

In June and July of 2008 15 female terrapins ranging from 605 g to 1438 g were captured on Great Bay Boulevard in Barnegat Bay, New Jersey. Great Bay Boulevard is the only road that travels through a peninsula of salt marsh in the southern most part of Barnegat Bay. This two way road stretches about 6.8 km, provides direct access to the bay on either side of the road, and is a site of high terrapin movement during the nesting season. During high tide, females search for suitable nesting areas often on the shoulder of the road. The females were collected by hand from the road. Between five mL and eight mL of blood were drawn immediately after capture using the subcarapacial venipuncture method described elsewhere (Basile et al. in prep). Plasma was separated within 2-6 hours after collection by centrifugation and was frozen in culture tubes at -80 ^{o}C. Two sub samples of plasma, 0.2 mL for thyroid hormone analysis and 0.5 mL for lysozyme assay, were aliquoted into 1 mL plastic tubes and frozen at -80 ^{o}C until shipped. Terrapins were transported in individual five gallon buckets to the field station in Waretown, NJ, where they were processed and induced. Terrapin processing included taking morphometric measurements, applying individual alphabetical scute codes and implanting passive integrated transponder (PIT) tags. After processing, these females were induced via interperotineal injection of Oxytocin as described elsewhere in (Sheridan et al., In Review). Eleven of the 15 terrapins laid their clutches. Clutch sizes ranged from 8 to 15 eggs with an

average clutch size of 11 eggs. All eggs were weighed and measured. One egg from 10 of these clutches was placed in a hexane-rinsed glass jar with a Teflon-coated lid and was frozen at -80 ^{o}C. An additional four eggs per clutch were used for an incubation study, and the remaining eggs were placed in a hatchery where they were released back to the bay after hatching. Females were returned to the bay close to where they were collected no more than 48 h after capture. *Thyroid Hormone Analysis*

Sub samples of plasma were sent to Texas A&M University in College Station, Texas and analyses were run by Duncan MacKenzie and Paul Licht. Thyroid hormone analysis was done by using Coat-A-Count Total T4 and Coat-A-Count Total T3 kits (Siemens, Los Angeles, CA), both of which quantify hormone concentration in diluted samples utilizing an antibody-coated tube method. The radio immuno-assay kits were donated by Dr. Scott Jaques, Director, Endocrine Analysis Laboratory, Texas Veterinary Medical Diagnostic Laboratory, College Station, TX.

Lysozyme Assay

Sub samples of plasma were sent to the Medical University of South Carolina and analyses were conducted by Margie Penden-Adams. Lysozyme activity was assessed using a standard turbidity assay described by Demers and Bayne (1997) with the slight modifications described in Day et al (2007). Briefly, hen egg lysozyme (HEL) was used as the standard curve in a 96-well plate assay. Terrapin plasma (25 µL/well) and lyophilized *Micrococcus lysodeikticus* were

added to wells in quadruplicate. Wells containing only plasma and buffer served as blanks. Absorbance at 450 nm measured with a spectrophotometer (SpectraCount; Packard, Meridian, CT) at five, ten, fifteen and twenty-five minutes and was subtracted from absorbance measured immediately (T0), and after correcting for the blank well absorbance which compensates for hemolysis or color variation in the samples. The resultant absorbance was converted to HEL concentration (micrograms per microliter) via linear regression of the standard curve.

Righting Response

The righting responses of 54 hatchlings were recorded. Claire Sheridan of Drexel University conducted these tests. The hatchlings were from a hatchery located at the field station in Waretown, New Jersey. They were from the same clutches used for the incubation experiments (Chapter 4) however were not in any way in contact with the experiment. Once the nest began to hatch the whole nest was uncovered and the hatchlings were placed in their own containers and kept organized by clutch. The righting response was tested after the yolk sac was completely resorbed within 4 to 5 days. No unnecessary handling of the hatchlings, including taking weights and measurements, was conducted until after the test was conducted. Hatchlings were allowed to acclimate to the container in which the test was to take place for 30-45 minutes. The testing environment was kept as quiet and controlled as possible. The test involved placing the hatchling on its carapace and timing how long it took for the terrapin to turn back over. If the

hatchling had not initiated effort to flip back at five minutes the response was recorded as a fail and the hatchling was assigned 300 seconds for the response time.

Calibration Solutions and Quality Control

Seven calibration solutions were prepared at differing concentrations that were extracted and cleaned up alongside samples. Internal standard solutions were added to samples prior to extraction. The compounds in the calibration solutions and internal standard solution are described in detail in Chapter 2. Three NIST SRMs were used as control samples. Three replicates of SRM 1957 (Organic Contaminants in Non-Fortified Human Serum) in addition to one replicate of an in-house control material of pooled loggerhead sea turtle plasma (Cc pool) were analyzed alongside the plasma samples. Three replicates of SRM 1947 (Lake Michigan Fish Tissue) were analyzed alongside the egg samples in addition to one replicate of an in-house control material of pooled loggerhead eggs (Cc comp). Laboratory procedural blanks and field blanks made from each lot of blood collection supplies were also analyzed.

Sample analysis

Plasma from the 15 live terrapins (between 2.7 g to 4.8 g) was extracted using focused microwave methods described in Chapter 2. A single pass of plasma extracts through an acidified silica column followed by an alumina column was sufficient. Eggs were mixed with diatomaceous earth, spiked with an internal standard solution of *iso*-octane, and extracted with DCM using

pressurized fluid extraction (Dionex Corporation, Sunnyvale, CA) according to Keller et al. (2004). These extracts were cleaned up using size exclusion chromatography with a 25 mm x 600 mm 10 um 100 Å PLGel column coupled to a 25 mm x 25 mm guard column (Polymer Laboratories) and also with acidified silica and alumina columns.

Lipid content was determined gravimetrically from initial extracts of plasma and eggs prior to any clean-up according to Keller et al. (2004). Final extracts (≈ 0.2 mL in volume) were injected twice on a gas chromatograph mass spectrometer (GC/MS; Agilent Technologies 6890N/5973 inert, Palo Alto, CA) and the limit of detection (LOD) was determined as described in Chapter 2.

Statistical Analysis and Calculations

For descriptive statistics and hypothesis testing statistics, all values below LOD were substituted with half the LOD, except zero was used when summing contaminant classes. All data were normally distributed. The statistical program SAS version 8.1 (SAS Institute Inc., Cary, NC) was used to test correlations between plasma POP concentrations and T4 plasma concentrations and lysozyme activity as well as between egg POP concentrations and righting response times in hatchlings. The average righting response for each clutch was correlated with the POP concentration that represented that clutch. Body condition index (BCI) was calculated as weight/ (straight line carapace length)^3 x 100,000 as described in Bjorndal et al (2000) for green sea turtles.

Results and Discussion

Terrapin plasma concentrations of all POPs reported in Table 1 and all eggs reported Table 2 have been reported and described thoroughly in previous terrapin studies (Chapters 1-3). Diamondback terrapin tissues, both plasma and eggs discussed in this current study, and fat, plasma and eggs discussed in previous studies show relatively moderate POP concentrations when compared to other turtle species (De Solla et al., 1998; Keller et al., 2004a; Moss et al., 2009; Basile et al., in Prep). We have determined that the environment of Barnegat Bay is not largely contaminated based on the lower to moderate POP levels measured in the terrapins and nesting beach sediments (Chapter 2). The congeners reported in Table 1 were either predominant congeners found in terrapin tissue or those that have been reported in other publications as possible endocrine disruptors such as PCB105, 153 and 156 and PBDE 100 (Vanbirgelen et al., 1995; Rudel et al., 2003). The ΣTCDD-like PCBs include PCBs 105, 118, 156 and157 and have been reported to have 2,3,7,8, tetrachlorodibenzo-p-dioxin (TCDD) like effects which include endocrine toxicity and immunotoxicity (Ahlborg et al., 1994). Congeners reported in Table 2 were congeners that are either predominant in terrapin eggs or those that have been shown to cause neurotoxicity like PBDEs 47, 99 and 153 (Eriksson et al., 1998; Viberg et al., 2003).

Plasma Thyroid Hormone Concentrations

Plasma concentrations of triiodothyronine (T3) and thyroxine (T4) were measured in 15 adult, nesting females. Due to very low concentrations and large

variability of T3 measured in only six of the turtles (mean 1.28 ng/mL, stdev 4.63 ng/mL) we did not statistically analyze those data (Table 3). The low concentrations of T3 were not surprising because detectable levels of T3 are rarely measured in turtles (MacKenzie, 2008). Plasma T4 concentrations ranged from 19.1 to 39.9 ng/ mL with an average concentration of 31.0 ng/mL and a standard deviation of 6.39 ng/mL (Table 3). Due to the slightly higher than expected levels of T4 in terrapin plasma, assay validations are currently being conducted to make sure there was not interference in the assay. Significant negative correlations were seen between female terrapin ΣPCB, ΣTCDD-like PCBs and ΣPBDE concentrations and total circulating thyroxine (T4) levels in the female plasma (Table 1, Figure 1). All individual PCB and PBDE congeners listed in Table 1 also had significant negative correlations to female plasma T4 levels. The strongest correlations were observed with PBDEs and the strongest was PBDE 100 (Table 1). At this point the correlations and conclusions in this chapter based on the T4 levels need to be understood as preliminary results until validation is complete.

The mechanism of PBDE and PCB (both parent compound and metabolite) induced thyroid hormone disruption is unknown but there are three commonly accepted hypotheses. First, the contaminants may induce enzymatic effects such as increased induction of uridine diphosphate glucuronyltransferase (UDPGT) that would cause increased biliary excretion of T4. Second, the contaminants may have a direct effect on the morphology of the thyroid follicles

thereby reducing the amount of T4 released. And third, hydroxylated metabolites of the contaminants may out compete T4 in binding to the thyroid hormone transporter protein transthyretin (TTR), thereby displacing T4 and allowing it to be more accessible for elimination (Kodavanti, 2005). The third hypothesis may be the most biologically significant and threatening. This is because other than decreasing the transport of T4 to the target organs the transporter protein TTR is now transporting toxic POPs to the target organ where they can elicit a toxic effect (Kodavanti, 2005). This example of endocrine disruption by a contaminant that in turn may also cause a neurotoxic effect demonstrates that chemicals labeled as endocrine disruptors are also likely to disrupt the immune system and elicit neurotoxicity. There is a strong and complex relationship between the endocrine and immune systems as well as complex interactions along the nero-endocrine-immune axis (Keller et al., 2006b). In this study the significant negative correlations between plasma POP concentrations and plasma T4 concentrations seen in wild terrapins may indicate that terrapin populations may be at risk for decreased survival and fitness as well as effects from disruptions in immunity and neurotoxicity.

Plasma Lysozyme Activity

Terrapin lysozyme activity in plasma of adult females is reported in Table 3. The activity was measured at four different time points with the 15 minute time point chosen as the optimum time for the assay. Positive significant correlations were seen between plasma lysozyme activity and female terrapin

plasma mirex, ΣPCB, ΣTCDD-like PCBs and ΣPBDE concentrations (Table 1, Figure 2). All other PCB and PBDE congeners reported in Table 1 except for PCB 180+193 and PBDE 100 also had significant positive correlations. The strongest correlation reported in Table 1 was with ΣTCDD-like PCBs followed by mirex.

We expected to see negative correlations between POP concentrations and lysozyme activity that would indicate immunosupression, rather we saw positive correlations suggestive of immunoenhancement (Table 1, Figure 2). Other studies looking at environmental contaminants and lysozyme activity generally report a decrease in activity. In diamondback terrapins in South Carolina, a significant decrease in lysozyme activity correlated with increases of total mercury concentrations in red blood cells (Blanvillain, 2005). In free ranging wild loggerhead sea turtles, lysozyme activity was significantly and negatively correlated with whole blood 4,4'- DDE and sum chlordanes (Keller et al., 2006a) and a positive correlation with mercury (Day et al., 2007).

Although our data are suggestive of immunoenhancement by POPs this may not be an advantageous effect. Disruption in the normal functioning of the innate immune system may be a very sensitive health endpoint for some reptiles such as alligators. In the American alligator, data suggests that the innate immune system components found in their serum is the main component responsible for keeping away infection (Merchant et al., 2003). Because they live in a wet environment and are often subject to boat injuries as well as intra-specific

competition that may cause injury, they often have open wounds. The antibacterial properties in their blood are extremely important for their health in the ecosystem in which they live. Terrapins live in similar ecosystems and are subject to threats such as high levels of boat traffic and collisions that may cause similar injuries, therefore the normal functioning of their innate immune system is just as important.

A speculative mechanism for immunoenhancement by endocrine disrupting, specifically estrogenic POPs, was proposed by Keller et al. (2006b). They suggested that the combination of three following factors may enhance immune functions at low level chronic exposure to estrogenic POPs. These factors are (1) the reported estrogenic and antiandrogenic activity of certain POPs, (2) the fact that the female of a species generally exhibits a stronger immune response that is caused by sex steroids and (3) that slightly higher than background estrogen levels may lead to enhanced immune functions (Keller et al., 2006b). A second hypothesis for immunoenhancement in terrapins at these environmentally relevant and low concentrations of POPs has to do with a possible hormetic dose-response which has been reported in other toxological studies (Calabrese and Baldwin, 2003). Hormetic dose-responses are defined as enhancement or suppression at a low concentration of an immune-disrupting chemical (in this studies context) with the opposite reaction at higher concentrations.

Even though terrapins seem to be experiencing immunoenhancement it is important to remember that any disruption in the normal functioning of a biological system may cause adverse effects. For example, auto-immune diseases and hypersensitivity are examples of immunoenhancement (Keller et al., 2006a).

Righting Response

The righting response of 54 hatchlings total, from 10 clutches, were recorded. The average time to right for all ten clutches ranged from 8.43 to 273 seconds. The shortest individual hatchling time to right was one second and the maximum time to right was >300 seconds. Out of all the compounds reported in Table 2 there was only one significant correlation. Terrapin egg concentrations of PBDE 47 were significantly and positively correlated with righting response (Figure 3). PBDE 99 had a strong correlation coefficient but was not significant at $p = 0.086$ (Table 2). The direction of these correlations was expected, indicating that it took the terrapins a longer time to right themselves with increased egg POP concentrations. Similar inverse relationships (longer time to right at greater contaminant concentrations) between righting response and PCB and PAH concentrations have been reported in snapping turtle hatchlings and mercury in red-eared sliders (Burger et al., 1998; Van Meter et al., 2006).

Although we did not see significant correlations for PCBs or any pesticides the significant correlation between PBDE 47 and righting response is intriguing. This is because PBDEs have been reported to disrupt neurobehavioral development in other species. For example, decreased spontaneous motor activity

and learning and memory function has been reported in mice as well as hypoactivity and the elimination of normal fright response causing higher predation rates in killifish (Eriksson et al., 1999; Timme-Laragy et al., 2006). Based on the significant correlation between PBDE 47 and righting response, hatchling terrapins may be at increased risk for predation, desiccation and overheating at higher concentrations of PBDEs.

From this study an interesting connection can be made between the effect of PBDEs on neurotoxicity and endocrine disruption. PBDEs at environmental levels showed disruption in thyroid hormones, specifically a decrease in terrapin plasma T4 levels as well as a decreased righting response indicating a disruption in neurobehavioral development in hatchling terrapins. One of the three possible mechanisms of thyroid hormone disruption discussed was the disruption in the transport of T4 to a target organ by TTR which is known to play an important role of transporting thyroid hormones through the blood brain barrier (Kodavanti, 2005). If PBDEs are crossing the blood brain barrier through transport by TTR they may be eliciting a neurotoxic effect.

Limitations

We tried to account for the limitations that accompany correlative studies which are twofold. First, in context to the thyroid hormones and lysozyme activity, is confounding factors, such as sex, body condition and hormones that can skew the true biological significance of the test. Because all plasma samples were collected from adult gravid females who were collected while trying to nest

and whose blood was collected immediately upon capture, we are relatively confident that sex and stress hormones can be ruled out as confounding factors. As for body condition, all terrapins outwardly appeared healthy with no signs of emaciation or injury and there were no significant correlations between plasma T4 concentrations and BCI (Table 1). There were however significant positive correlations between plasma lysozyme activity and body condition index (Table 1). This data suggests that lysozyme activity increases with "healthier" turtles. In context to the righting response, confounding factors such as testing temperature, egg size, egg incubation temperature, and clutch identity may cause among-individual variation in righting response (Steyermark and Spotila, 2001). These factors were tested in juvenile snapping turtles and only testing temperature and clutch identity affected righting response (Steyermark and Spotila, 2001). For the terrapin study the testing environment was kept as controlled as possible for temperature, light and noise so we feel confident that there was not an influence of differing testing temperatures or other testing environment stressor. This study extends the finding of clutch identity to chemical exposure among clutches, and suggests that PBDE 47 may be one reason why Steyermark and Spotila (2001) saw differences across clutches.

The second set of limitations is caused by the use of multiple individual correlations and the probability of certain outcomes that may not be biologically significant. By chance we would expect five percent of the correlations in Tables 1 and 2 to be significant. However, 52% of the correlations were significant in

Tables 1 and 2. We would also expect half of those significant correlations to be positive and the other half negative again based on probability. All significant correlations in Table 1 were negative for T4 and positive for lysozyme activity. A third set of limitations is caused by co-correlations between individual compounds and compound classes. In this study there were significant correlations between compound classes, the strongest between PCBs and PBDEs in plasma. This may indicate that a significant correlation seen in one compound, for example 4,4'-DDE may actually be driven by another compound such as a PCB. In an environmental study there is no way to control for this type of confounding factor. These results then may be considered preliminary but are still important because we observe a relationship between POPs and the health endpoint response. Further controlled tests can be conducted to provide more data supporting the hypotheses that environmental concentrations of reported POPs are causing disruption in the immune, endocrine and neurobehavioral systems.

Conclusions

In this study we have provided data suggesting that terrapins may be at risk for immune and endocrine disruption (preliminary, awaiting data validation) at environmentally relevant concentrations of mirex, PCBs and PBDEs as well as neurotoxicity (PBDEs only). The connection we observed between significant correlations with PBDEs to both endocrine disruption and neurotoxicity endpoints strengthens the idea that a chemical that disrupts one physiological system has the potential to affect others based on the complex interactions along the neuro-

endocrine-immune axis. Another consideration must also be realized when introducing the idea of complex interactions. In wildlife studies, organisms will inevitably have been exposed to many different chemicals and unless you are sampling a site with a known source of pollution you cannot control for the amount of exposure to a specific contaminant in your study organism. Therefore the correlations observed between individual contaminants and congeners may also be due to synergistic, additive or antagonistic effects. Synergistic effects of mixtures of contaminants have often been reported to have a more pronounced effect than an individual compound (Bergeron et al., 1994; Burton et al., 2002).

The diamondback terrapin has previously been reported to be indicator for monitoring POPs in the estuarine ecosystem (Kannan et al., 1998; Keller et al., 2006b; Blanvillain et al., 2007; Basile et al., in Prep). Because we are seeing associations between terrapin POP concentration and certain health indicators they may also be useful as bioindicators for endocrine disruption and immunotoxicity within the estuarine environment in respect to other species and humans. Further studies would however be necessary for validation for the use of terrapins for bioindicators of these health effects. In the current study only one measure of each health endpoint system was analyzed. In addition to controlled cause and effect laboratory experiments validation can be achieved through more comprehensive testing of each of the systems discussed. To validate the hypothesis that terrapins are at risk for POP induced immunotoxicity a set of tests called the Comprehensive Screening Set as suggested by Keller et al. (2006b)

could be used. This set of tests includes measuring lysozyme activity but also includes tests that measure cell mediated immunity, humoral immunity and integrated immunity, all of which can be accomplished using wild animals that can be re-released. Validation of endocrine disruption can be accomplished by testing other aspects of the endocrine system such as POP induced effect on sex hormones and stress hormones. The neurobehavioral exam protocol suggested by Chrisman et al. for use in assessing neurologic abnormalities in sea turtles that may cause detrimental effects in wild turtles can be adapted to terrapin behavior and used to validate POP induced neurotoxicity (1997).

Table 1. Summary of persistent organic pollutant concentrations in terrapin plasma and their associated Pearson Correlation Coefficients for two different health parameters. Asterisks denote significant coefficients at $p < 0.05$. Coefficients in bold are Spearman Correlation coefficients.

	Terrapin Plasma (ng/g wm) n=15			Correlation Coefficients				
Compound	**Mean**	**Stdev**	**n>LOD**	**Plasma T4 Conc.**	**Lysozyme Activiy @ 5min**	**Lysozyme Activiy @ 10min**	**Lysozyme Activiy @ 15min**	**Lysozyme Activiy @ 25min**
Oxychlordane	0.153	0.078	15	-0.3132	0.3327	0.3266	**0.2811**	0.3103
Mirex	0.068	0.085	7	0.0376	**0.7785***	0.4759	**0.6034***	0.4352
4,4'-DDE	0.397	0.210	15	-0.1497	0.3080	**0.1966**	**0.2775**	0.2527
ΣDDTs	0.425	0.224	15	-0.0713	0.3024	**0.2306**	**0.3151**	0.2385
PCB 99	0.384	0.267	15	-0.3127*	0.6318*	0.6045*	0.5927*	**0.5964***
PCB 105	0.212	0.158	15	-0.5970*	NR	NR	NR	NR
PCB 118	0.732	0.524	15	-0.6640*	0.5741*	0.5594*	**0.5443***	0.4930
PCB 138	0.648	0.408	15	-0.5821*	0.5454*	0.5008	**0.5515***	0.4456
PCB 153+132	2.24	1.66	15	-0.6606*	0.6311*	0.6050*	0.5779*	0.4972
PCB 156	0.055	0.037	15	-0.6613*	NR	NR	NR	NR
PCB 180+193	0.432	0.301	15	-0.6005*	0.6396*	**0.2431**	**0.2256**	0.4340
ΣPCBs	6.23	4.38	15	-0.6667*	0.6279*	0.5851*	**0.5139***	0.4831
ΣTCDD-like PCBs	1.02	0.738	15	-0.6223*	0.5861*	0.5703*	**0.6142***	0.4951
PBDE 100	0.016	0.020	8	-0.7001*	0.4736	0.4332	0.4239	0.3472
PBDE 154	0.051	0.034	15	-0.5180*	0.5544*	0.5025*	0.5028*	0.4613
PBDE 153	0.009	0.009	9	-0.6924*	0.5028*	0.5407*	0.5481*	0.52059*
ΣPBDEs	0.088	0.082	15	-0.6097*	0.6253*	0.5705*	0.5603*	0.4865
% Lipid	0.447	0.193	15	-0.1150	0.4146	0.3342	0.4261	0.4042
BCI	17.0	1.44	15	0.0610	0.6258*	0.6281*	0.6302*	0.5205*
Mass	979	222	15	**-0.3536**	0.2206	**0.1448**	**0.1164**	0.2103
CL	175	14.2	15	-0.2905	-0.1878	-0.2625	-0.3363	-0.3376

ΣTCCD-like PCBs include PCB 105+118+156+157

Table 2. Summary of persistent organic pollutant concentrations in terrapin eggs and their associated Pearson Correlation Coefficients for righting response. Asterisks denote significant coefficients at $p < 0.05$.

	Egg ng/g wm n=10			Correlation Coefficient
Compound	**Mean**	**Stdev**	**n>LOD**	**Righting Response**
Oxychlordane	3.68	2.01	10	-0.2362
Mirex	0.232	0.133	10	-0.3031
4,4'-DDE	8.17	3.76	10	-0.2322
ΣDDTs	8.26	3.76	10	-0.2274
PCB 99	5.33	2.13	10	-0.2261
PCB 118	9.26	4.45	10	-0.2115
PCB 138	6.15	2.80	10	-0.2000
PCB 153+132	24.7	12.8	10	-0.1030
PCB 180+193	3.91	2.65	10	-0.1602
ΣPCBs	72.4	34.8	10	-0.1915
ΣTCDD-like PCBs	12.7	6.17	10	-0.5467
PBDE 47	0.103	0.124	10	0.6419*
PBDE 99	0.072	0.040	10	0.5686
PBDE 154	0.104	0.027	10	-0.1563
PBDE 153	0.123	0.073	10	-0.2107
ΣPBDEs	0.608	0.253	10	0.1515
% Lipid	6.05	1.40	10	-0.0709
Egg Mass	8.03	1.40	10	-0.0330

Table 3. Summary of plasma thyroid hormone levels and plasma lysozyme activity in female diamondback terrapins of Barnegat Bay, New Jersey.

Turtle	Plasma T3 Conc. (ng/mL)	Plasma T4 Conc. (ng/mL)	Lysozyme Activiy @ 5min (µg HEL/ µl)	Lysozyme Activiy @ 10min (µg HEL/ µl)	Lysozyme Activiy @ 15min (µg HEL/ µl)	Lysozyme Activiy @ 25min (µg HEL/ µl)
18837	0	31.9	1.58	1.50	1.43	1.47
18846	0	36.5	1.58	1.64	1.81	1.51
18864	0.01	35.5	2.59	2.21	2.40	2.34
18875	0	19.1	2.08	1.99	2.08	1.86
18883	0.06	29.8	3.39	3.32	3.61	3.58
18892	18	27.0	1.47	1.68	1.95	1.72
18893	0	38.9	1.98	1.81	2.08	1.79
18916	0.03	33.8	1.37	1.72	2.20	2.18
18926	0	21.7	4.20	3.99	3.85	3.42
18934	0	27.5	2.69	1.95	2.23	1.96
18936	0	22.3	2.18	2.04	2.20	2.14
18939	0	39.9	1.47	1.37	1.46	1.37
18941	1	33.0	1.98	2.21	2.37	2.32
18944	0.04	31.3	2.59	2.61	2.35	2.42
18941B	0	36.8	1.37	1.24	1.46	0.98
Mean	1.28	31.0	2.17	2.09	2.23	2.07
Stdev	4.63	6.39	0.813	0.739	0.693	0.707
Median	0	31.9	1.98	1.95	2.20	1.96
Min	0	19.1	1.37	1.24	1.43	0.976
Max	18.0	39.9	4.20	3.99	3.85	3.58

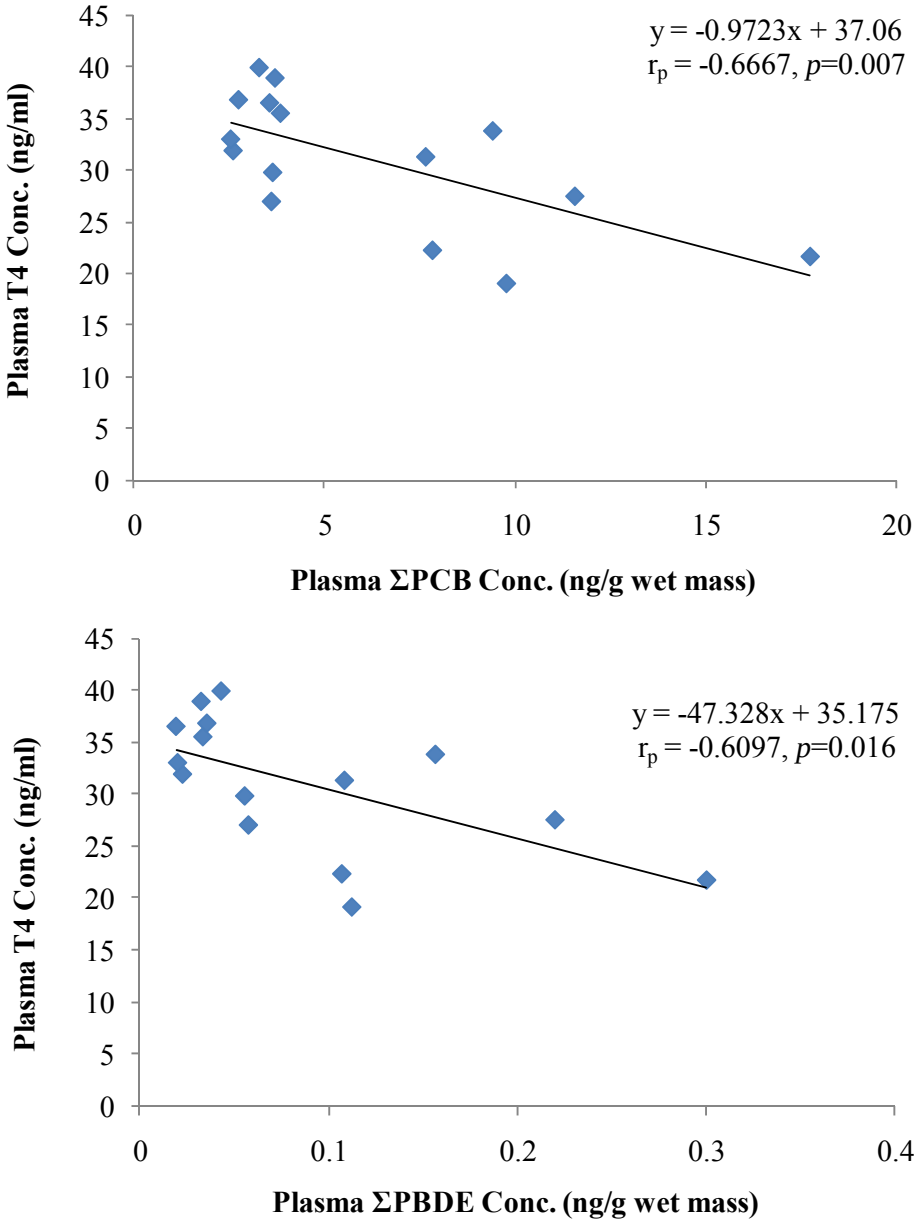

Figure 1. Representation of the statistically significant correlations between female terrapin plasma POP concentrations and T4 plasma concentrations in terrapins in Barnegat Bay, NJ. Pearson correlation coefficients (r_p) with associated p-value are given.

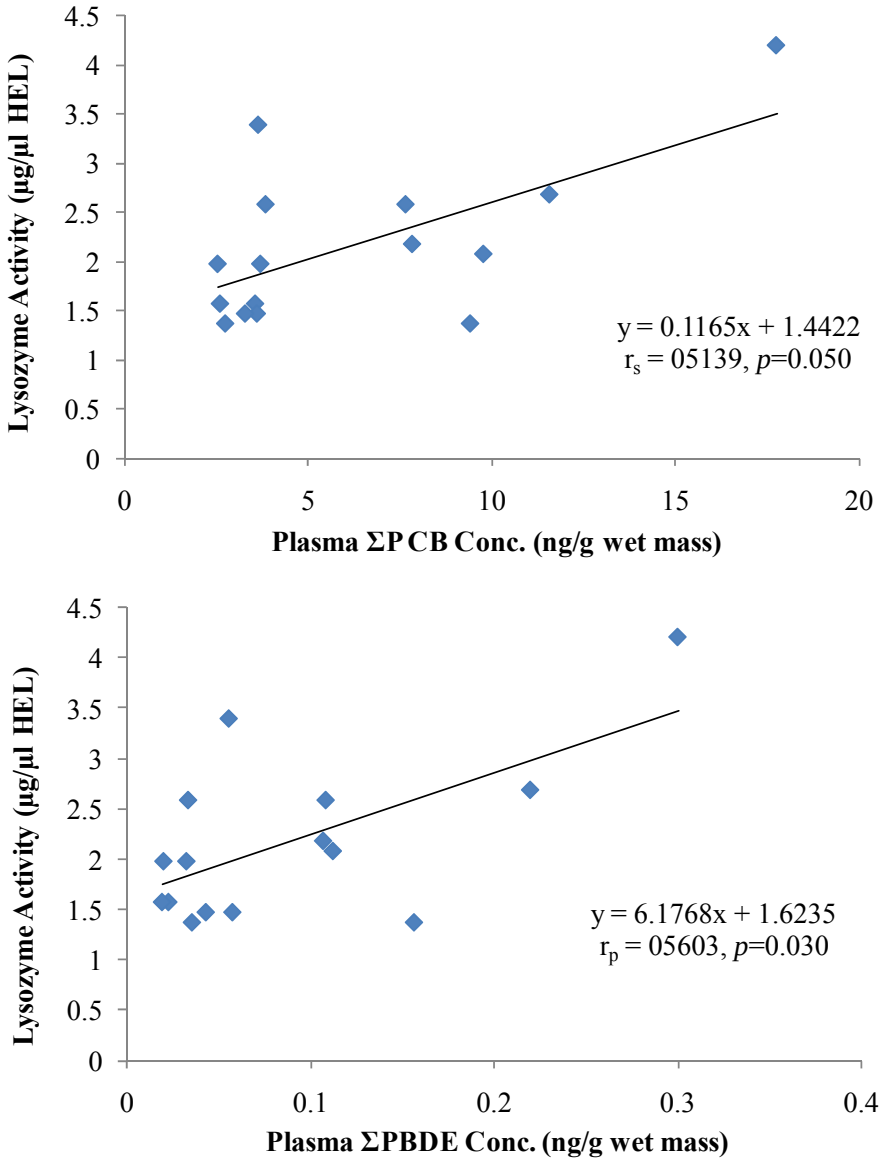

Figure 2. Representation of the statistically significant correlations between female terrapin plasma POP concentrations and lysozyme activity in terrapins in Barnegat Bay, NJ. Pearson correlation coefficients (r_p) with associated p-value are given.

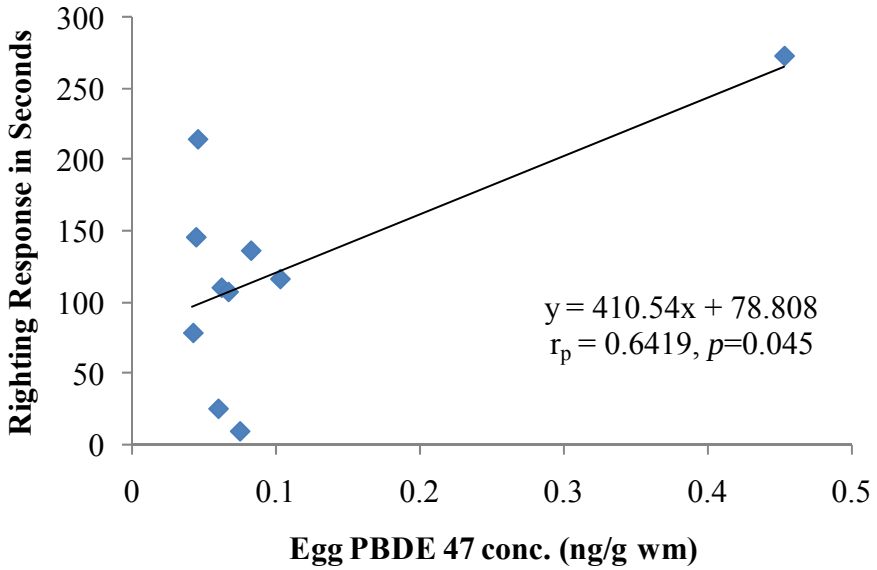

Figure 3. Representation of the statistically significant correlations between terrapin egg PBDE 47 concentrations and righting response in Barnegat Bay, NJ. Pearson correlation coefficients (r_p) with associated p-value are given.

CHAPTER 6:
CONCLUSIONS, FUTURE CONSIDERATIONS AND MANAGEMENT SUGGESTIONS

CONCLUSIONS

CHAPTER 2: *Diamondback Terrapins as Indicator Species of Persistent Organic Pollutants: Using Barnegat Bay, New Jersey as a Case Study*

1. Diamondback terrapins are good bioindicators of organic contamination in estuarine environments and are indicators of local contamination.
2. Collecting plasma is non-lethal, relatively easy to collect and represents the stored contamination burden.
3. A single terrapin egg from a nest represents the POP contamination of the clutch, which can reduce the impact of sampling on terrapin populations.
4. The terrapin population of Barnegat Bay has levels of organic contaminants on order with other wildlife species.
5. Barnegat Bay terrapins exhibit an atypical PBDE contamination pattern in their tissues and eggs.
6. Terrapins may be capable of biotransformation and/or metabolism of PBDE 47 and PBDE 99.

CHAPTER 3: *Persistent Organic Pollutants in Gravid Diamondback Terrapin Plasma, Eggs and Nesting Sediments in Barnegat Bay, NJ*

1. Maternal transport was demonstrated simply by the presence of POPs in eggs that had never touched sediments.
2. No significant correlations were observed between female terrapin plasma POP concentrations and her eggs.
3. There is a spatial variation of POP concentrations in Barnegat Bay terrapin tissues where northern sampling sites in general are more contaminated than southern sampling sites.
4. Interesting contamination pattern for sum PBDEs, where PBDE concentrations were low in the northern sites sampled above Toms River but increased just below Toms River and stayed elevated in the rest of the southern sampling sites. Possible deca-PBDE contamination from textile coating plant in Toms River watershed.

CHAPTER 4: *Transfer of Polybrominated Diphenyl Ethers (PBDEs) and Other Persistent Organic Pollutants (POPs) from Nesting Sediments to Developing Diamondback Terrapin Eggs*

1. There is low but measureable transfer of PBDEs (0.0001 to 1.7%), PCBs (0.302 to 2.22%), mirex (0.118 to 0.566%) and DTT metabolites (0.0009 to 0.930%) from natural sandy nesting sediments into eggs with developing terrapin embryos. The percent transfer does not reflect the amount transferred into the embryo, only what has passed through the egg shell.

2.Lower brominated PBDEs had greater transfer as was expected based on the higher bioavailability reported for those compounds in the literature. Lower chlorinated PCBs also had greater transfer.

3.The percent transfer calculated using a mass balanced equation may underestimate full potential for a contaminant to pass through the egg shell in natural nesting environments.

4.A developing egg facilitates a greater percent transfer then non-developing eggs, most likely due to ability of POPs to volatilize and pass through egg shell along with oxygen and carbon dioxide during gas exchange.

5.Measured percent transfer of PBDEs and other POPs in natural and non-spiked sediment was determined to be unsubstantial and that maternal transfer is the greatest source of POPs to developing embryos.

6.Although percent transfer of POPs was determined to be unsubstantial there is a risk for eggs developing in highly contaminated sediment. As little as a three percent transfer may increase the eggs internal concentration enough to cause toxic effects.

7.This experiment provided valuable information about the volatility of PBDEs from sediments.

CHAPTER 5: *Examination of Endocrine Disruption, Immunotoxicity and Neurotoxicity by Persistent Organic Pollutants at Environmental Concentrations in Diamondback Terrapins of Barnegat Bay, NJ.*

1. Significant negative correlations between gravid female terrapin plasma POP concentrations and plasma T4 suggest the terrapin population in Barnegat Bay may be at risk for decreased survival and fitness from endocrine disruption at low POP concentrations.
2. Significant positive correlations between gravid female terrapin plasma POP concentrations and plasma lysozyme activity suggest that the terrapin population of Barnegat Bay may be experiencing immunoenhancement at low concentrations of POPs..
3. A significant positive correlation between terrapin egg PBDE 47 concentrations and righting response suggests that developing terrapin embryos may be experiencing disruptions in neurobehavioral development therefore putting hatchling terrapins at risk for predation, desiccation and overheating as well as behavioral and memory deficits.
4. Terrapins may be useful as bioindicators for endocrine disruption and immunotoxicity within the estuarine environment in respect to other species and humans.

FUTURE CONSIDERATIONS:

Biomagnification and Metabolism/Biotransformation of PBDEs

The biomagnification factor calculated for PBDE 47 for terrapins and blue mussels, a possible terrapin prey item, was less than one. We expected to see a BMF of greater than one based on the general bioavailability of PBDE 47 in wildlife samples, its octanol water partition coefficient and what has been reported in the literature. We suggested that the BMF calculated is suggestive of a possible biotransformation or metabolism of PBDE 47. Although a BMF was not calculated for PBDE 99 (not detected in prey) we suggest that the same may occur for PBDE 99 because it is measured in lower than expected levels in terrapins tissues similarly to PBDE 47. A more in depth examination of preferred terrapin diet species as well as their POP concentrations in Barnegat Bay should be considered. To assess how terrapins biotransform these compounds exposure studied would be needed.

Maternal Transport

Although we have data validating the occurrence of maternal transport of POPs from maternal tissues to offspring we did not observe significant correlations between POPs measured in maternal plasma and egg contents which was unexpected. We suggest that collecting plasma, a short term storage depot for POPs, at the time of ovoposition may not reflect the POPs associated with the lipids at the time of yolking. A more in depth examination of the seasonal

variation of POP patterns in terrapin plasma based on diet and reproduction should be considered.

Spiking and Transfer Studies

The methods used for the transfer study ultimately provided the data we anticipated however we can suggest some changes in methodology that may allow for less confounding factors. For example, we did not anticipate there to be cross contamination within the incubator. Therefore in future experiments PBDE spiked sediments should be placed in a separate incubator equipped with air scrubbers as to not contaminate the air in going into the other incubator. During sediment preparation steps we may have altered the background POP contamination patterns and levels due to baking sediments to get rid of water at a high temperature (165 $^{\circ}$C). In the future lower temperatures should be used to dry sand. One last consideration must be mentioned when doing a spiking experiment. The actual concentration post spiking should always be measured. We found that during the sediment preparation after spiking, nearly 40% of the original dose of PBDEs had evaporated along with the solvent or had just volatilized. If we had not calculated the actual sediment concentrations the calculations would have been extremely underestimated.

Health Endpoints

Although we observed associations between POP concentrations in terrapin plasma and hatchlings with certain health endpoints we only used one test each to measure three physiological systems. Because the data in this dissertation

suggest terrapins may be experiencing POP induced effects associated with their immune systems, endocrine systems and neurobehavioral development it would be beneficial to a management plan (as discussed below) to incorporate a larger suite of tests to measure effects in each of the three systems. The tests are briefly mentioned in Chapter 5.

To provide a more comprehensive measure of immune function in terrapins more than one aspect of immunity should be assessed. Keller et al. suggests a suite of twelve tests called the Comprehensive Screening Set, specifically adapted for reptiles that measures innate, cell mediated, humoral and integrated immunity (2006b). All of these tests can be conducted in wild animals non- lethally and do not require captivity, recapture or compromising or altering their immune systems to obtain the results. This set of tests was complied by adapting a two tier testing scheme used by the National Toxicology Program for testing in lab rodents and the three tier testing scheme for wildlife risk assessment in fish, birds and mammals (Luster et al., 1988; Weeks et al., 1992).

To provide a more comprehensive measure of endocrine disruption more than just thyroid hormones can be considered. Endocrine disrupting chemicals can elicit a toxic effect through both direct (agonistic or antagonistic) and indirect (influencing hormone metabolism) interactions with hormone receptors (Lintelmann et al., 2003). Many POPs have been implicated in alteration of sex-hormones or have been reported to mimic sex hormones. For example PCBs have been reported to act as environmental estrogens in the red eared slider (Bergeron

et al., 1994). Because the endocrine system as a whole can be very diverse it is often easier to study a certain subset, like thyroid hormones, especially if the contaminant in question is known to cause thyroid hormone disruption. Neurotoxicity encompasses a broad system of physiological systems. In this dissertation only neurobehavioral development was assessed. To complete this assessment we used hatchling terrapins which only told us about possible disruptions in development. In addition to developmental tests for neurotoxicity in hatchlings, adult or sub adult terrapins should be examined to determine if the effects were permanent or if they are even noticeable at an advanced age after they have had time to accumulate higher levels of compounds. Chrisman et al. has adapted a neurologic exam for sea turtles used originally to assess cats and dogs that complies a checklist of about 40 different tests and observations in live turtles (1997). This suite of tests can be easily adapted for terrapins.

MANAGEMENT

The Barnegat Bay- Little Egg Harbor estuary like all other estuaries is an extremely highly productive and important habitat. It is home to many environmentally sensitive habitats such as waterfowl nesting grounds, salt marshes, submerged aquatic vegetation beds, finfish nurseries and shellfish beds (BBEP et al., 2002). In 1995, under an amendment to the Clean Water Act, the Environmental Protection Agency (EPA) enlisted Barnegat Bay into the National Estuary Program where grant money is provided to manage estuaries threatened

by pollution, land development and overuse. The primary management issues in Barnegat Bay include nutrients, conventional pollutants (basically those which are treated or found in waste water), pathogens, human population growth, habitat loss/ alteration, species loss/ decline, fisheries loss/decline, introduced/pest species, freshwater inflow and drinking water problems (USEPA, 2010). Because of the impaired state of the Bay there has been an extensive characterization report prepared as well as a monitoring program plan. The highest priority problem in Barnegat Bay that receives the most focus publically and scientifically is nutrient and organic loading to the Bay since it is classified as a moderately eutrophic system (BBNEP, 2001).

Although eutrophication is a serious problem that must be attended to, there are other issues that may have the potential to affect estuarine species population and community structures just as severely. The EPA describes non-point source pollution as being the most important issue when addressing the issues surrounding water quality and the health of the living resources of the Bay (USEPA, 2010). Currently, the most extensive monitoring of biotic reactions to environmental stress occurs in the freshwater tributaries in the Barnegat Bay watershed. The New Jersey Department of Environmental Protection (NJDEP) initiated a biomonitoring program in 1992 called the Ambient Biomonitoring Network (AMNET). The goal of this program was to evaluate the biological integrity of aquatic invertebrate communities in freshwater streams and tributaries (BBNEP, 2001). This is a statewide network that collects data on five metrics

that together will assess the condition of the benthic macro invertebrate communities every five years. There are 61 sites monitored within the Barnegat Bay watershed. The metrics collected within a site are compared to a statewide reference database and then are given a community impairment score. Only one of the five metrics, species richness, may be indicative of environmental stress caused by toxic contaminants. The reason that benthic macro invertebrates are used for biomonitoring is because they are sensitive to minor changes in water quality and therefore are useful in indicating a wide range of environmental disturbances. In the context of persistent organic pollutants, unless there is a sudden point source of contamination, this type of biotic index may not indicate any risk of environmental stress with regards to POPs concentrations in the sediments or biota. This is because benthic macro invertebrates, although they are sensitive organisms, they are short lived. POPs are chemicals that cause environmental stress in wildlife through accumulated low level chronic exposure that would only be noticed in a longer lived higher tropic level species.

AMNET in cooperation with a surface water quality monitoring network conducted by the United States Geological Survey (USGS) is the basis for state water quality inventory that is reported to the United States Environmental Protection Agency (USEPA). These two monitoring networks are also the foundation for statewide water quality planning and management decisions. These programs are effective in monitoring water quality parameters of the freshwater that enters the Bay such as nutrient loading but exclude information on persistent

organic pollutants. The freshwater entering the Bay from tributaries is where the contaminant loads originate whether through one or a combination of non-point and point sources such as atmospheric deposition, groundwater pollutant transfer, suburban and construction run-off, industrial waste water discharge, marinas and dredged material. The Barnegat Bay National Estuary Program Characterization Report has addressed the issue of organic contaminants (which includes POPs) as being poorly characterized and that their effects in organisms has not been addressed (BBNEP, 2001). Perhaps a third monitoring program should be included in water quality planning and management decisions, one that addresses contaminants in the Bay and the risks to Bay organisms and human health.

Utilizing an indicator species that is capable of monitoring the accumulation of contaminants through low level chronic exposure, as well as monitoring certain health biomarkers, would address the characterization of contaminants entering the Bay as well as the effects and possible health risks to wildlife species inhabiting the sensitive estuarine environments and human health. In this dissertation data have been provided suggesting that the diamondback terrapin is a useful indicator species of persistent organic pollutants in the estuarine ecosystem and that endocrine disruption, immunotoxicity and neurotoxicity associated with these contaminants may be able to be monitored through the terrapin. By simply collecting non-lethal terrapin plasma samples from individuals representing specific sampling locations throughout the bay we can characterize the contamination pattern and levels both locally and throughout

the Bay. We may also be able use terrapin plasma to indicate risks of endocrine and immune disruption and neurotoxicity based on the environmental contaminant levels collected which can indicate similar health endpoints that may cause effects in other species such as endangered and threatened species as well as humans.

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Appendix 1

Raw data for pre and post incubation PBDE concentrations in spiked sediments. Percent difference between day six and day ten sediment concentrations are also reported. A negative % difference denotes loss of PBDEs from day six to day 10.

	PRE Incubation Sediments ng/g dry mass						POST Incubation sediments ng/g dry mass						% Difference between day 6 and day 10 for Pre-Incubation Sediment	
Treatment	SPIKED SAND			SPIKED DREDGE			SPIKED SAND			SPIKED DREDGE			SPIKED SAND	SPIKED DREDGE
Day	6	9	10	6	9	10	6	9	10	6	9	10		
30	<0.108	<0.120	<0.120	<0.114	<0.124	<0.105	<0.107	<0.111	<0.114	<0.097	<0.124	<0.117	N/A	N/A
17	<1.03	1.60	0.734	<1.08	0.966	0.387	<1.02	2.00	0.354	<0.920	1.18	0.457	59.4	20.7
25	2.17	<1.58	<0.611	<1.64	0.659	<0.535	1.65	<1.60	<0.582	1.83	<0.633	<0.600	21.1	178
33+28	4.26	3.70	1.33	4.36	2.00	0.960	3.06	3.00	0.600	4.92	2.42	2.03	-0.776	27.9
75	<0.657	<0.666	0.707	<0.691	<0.156	0.753	<0.649	<0.674	0.619	<0.587	0.426	2.13	-71.5	239
49+71	<0.386	<0.392	5.28	12.5	<0.046	3.43	<0.382	13.3	2.48	<0.346	7.61	4.74	96.9	-22.2
47	664	612	354	905	328	238	531	595	175	875	401	346	-5.28	10.2
66	5.47	5.28	1.91	5.25	2.51	1.97	5.07	4.20	1.02	6.96	3.77	2.05	-1.91	31.2
100	294	279	177	377	171	120	238	256	87.9	400	212	171	-9.06	17.2
119	1.57	<1.09	1.95	<1.13	0.613	0.629	<1.06	<1.10	0.844	<0.962	0.808	2.19	-71.7	142
99	919	871	406	1250	383	283	731	810	213	1240	465	435	-1.52	11.6
116	<0.348	<0.353	<0.489	<0.366	<0.508	<0.428	<0.344	<0.357	801	<0.311	<0.507	<0.480	N/A	N/A
85+155	33.3	31.1	10.6	28.3	13.2	9.57	25.6	29.6	6.07	37.7	15.0	11.1	7.00	24.9
154	43.4	40.9	17.9	40.8	19.3	15.5	33.8	36.4	11.3	54.4	22.9	20.0	-2.58	28.6
153	42.6	39.2	16.4	40.0	16.5	15.1	31.1	35.2	10.9	51.6	19.6	19.3	-3.84	26.4
138	4.83	4.12	1.54	3.22	1.80	1.42	3.65	2.96	1.04	5.27	1.95	1.50	-7.17	35.3
156	<0.793	<0.804	0.115	<0.835	<0.041	<0.034	<0.784	<0.813	<0.037	<0.709	<0.041	<0.039	N/A	N/A
183	12.4	9.64	0.544	19.6	0.650	0.465	9.99	10.2	0.363	13.1	0.842	0.548	35.1	-30.2
191	0.712	0.771	0.351	2.45	0.130	0.142	1.16	<0.259	0.315	0.444	0.132	0.314	-26.7	-67.3
181	0.840	<0.268	0.201	0.000	0.122	0.118	<0.262	<0.271	<0.035	<0.237	0.175	0.276	N/A	87.9
190	1.36	<0.185	0.000	0.000	0.229	<0.033	<0.180	<0.187	0.264	1.58	0.332	<0.037	-81.5	733
203	2.53	2.04	0.951	3.46	0.768	0.567	2.16	2.02	0.812	1.82	1.02	0.768	21.8	-24.6
205	<0.233	<0.237	<0.023	<0.246	<0.024	<0.020	<0.231	<0.240	<0.021	<0.209	<0.023	<0.022	N/A	N/A
206	590	604	299	442	302	307	412	775	304	512	276	406	-2.80	13.7
209	17200	16600	7720	21800.00	9300	7440	14300	15100	5190	23500	11600	8510	0.462	13.1
∑PBDEs	19800	19100	9020	24900	10500	8440	16300	17800	6810	26700	13100	9930	1.91	13.0

N/A = not applicable because not measured on one of days.

VITA
Emily R. Basile

EDUCATION

2010 Ph.D Environmental Science. Drexel University, Philadelphia, PA,
Expertise: Ecotoxicology, Estuarine Ecology and Conservation Biology
Dissertation Title: *Persistent Organic Pollutants in Diamondback Terrapin (Malaclemys terrapin) Tissues and Eggs and Sediments from Barnegat Bay, New Jersey.*

2004 Marine Science Consortium, Wallops Island, VA

2005 B.S. Biology. Millersville University, Millersville, PA,
Concentration: Biology and Marine Biology
Thesis: *Seasonal Distribution of native blue crabs (Callinectus sapidus) and invasive green crabs (Carcinus maenas) in the salt marsh creeks in Stone Harbor, NJ*.

2000 Semester at Sea Summer Program, Franklin Marshall College, NH
Concentration: Ocean Sciences

ACADEMIC & RESEARCH EXPERIENCE

2001	Educational and Aquarium Intern at Wetlands Institute, Stone Harbor, NJ
2003-2005	Undergraduate Science Tutor, Millersville University
2003-2005	Ecology and Cephalopod Lab Assistant, Millersville University
2005	Undergraduate Research, Millersville University
2005	Research Assistant, Drexel University (Sea Turtle Nesting Ecology, Costa Rica)
2006-Present	Teaching Assistant, Drexel University
2006-2009	Graduate Research, Drexel University
2006-2009	Research and Volunteer Coordinator, Drexel University (Earthwatch Institute Project: Diamondback terrapins of Barnegat Bay, NJ)
2007-2009	Graduate Research, Drexel NIST, Analytical Chemistry Lab, Charleston, SC)

PRESENTATIONS

2005 Atlantic Estuarine Research Society, Solomons, MD (Poster Presentation)

2007 Hudson Delaware Regional Chapter SETAC meeting (Poster Presentation)

2009 SETAC North American 30th Annual Meeting, New Orleans, NA (Oral Presentation)

2010 *Seminar* at the Patrick Environmental Center, ANS in Philadelphia.

2010 AERS Spring Conference, Atlantic City, NJ (Poster Presentation)

2010 *Guest Lecture* at Drexel University for Dr. Susan Kilhams' graduate Aquatic Toxicology Class.