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High incidence of deformity in aquatic turtles in the John Heinz National Wildlife Refuge

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This paper presents findings on the prevalence of developmental abnormalities in turtles at a national wildlife refuge that have direct relevance to studies on the effects of contamination on development and morphology of vertebrates.

Abstract

The John Heinz National Wildlife Refuge is subject to pollution from multiple sources. We studied development of snapping turtle (*Chelydra serpentina*) and painted turtle (*Chrysemys picta*) embryos from the refuge from 2000 through 2003. Mean annual deformity rate of pooled painted turtle clutches over four years ranged from 45 to 71%, while that of snapping turtle clutches ranged from 13 to 19%. Lethal deformities were more common than minor or moderate deformities in embryos of both species. Adult painted turtles had a higher deformity rate than adult snapping turtles. Snapping turtles at JHNWR had high levels of PAH contamination in their fat. This suggests that PAHs are involved in the high level of deformities. Other contaminants may also play a role. Although the refuge offers many advantages to resident turtle populations, pollution appears to place a developmental burden on the life history of these turtles.

Keywords: Deformity; Development; *Chelydra serpentina*; *Chrysemys picta*; Wildlife refuge; PAHs

1. Introduction

The urban setting of the John Heinz National Wildlife Refuge (JHNWR) in Philadelphia, Pennsylvania made the refuge subject to pollution from many sources such as: (1) runoff from adjacent highways and railroads, (2) atmospheric deposition from the city and the neighboring airport, and (3) seepage from various industrial and municipal sites along the Lower Darby Creek watershed. The JHNWR included a large, shallow, man-made impoundment fed by Darby Creek, a freshwater tidal creek. The impoundment provided abundant food, basking sites and refugia for large numbers of snapping turtles (*Chelydra serpentina*), painted turtles (*Chrysemys picta*), slider turtles (*Trachemys scripta*), redbellied turtles (*Pseudemys rubriventris*), and stink pot turtles (*Sternotherus odoratus*). Nesting sites were abundant in the 486 ha refuge.

The Darby Creek watershed includes six contaminated sites, including an oil tank farm, two landfills, a retired sewage treatment plant, a retired municipal incinerator, and an industrial property. Polycyclic aromatic hydrocarbons (PAHs) such as benzo(*a*)pyrene and dibenzo(*a*)anthracene, polychlorinated biphenyls (PCBs), metals such as arsenic, copper, cadmium, mercury and lead, and assorted volatile and semivolatile organic compounds seeped into Darby Creek from the landfills. Therefore, the United States Environmental Protection Agency (USEPA) officially added the landfills to its National Priorities List of contaminated sites (NPL) in 2001 (U.S.E.P.A., 2001, 2003). Contamination from Darby Creek apparently entered the impoundment through inflow from the creek and the transfer of water and sediment into the refuge during floods from large storms and hurricanes. Recent analyses indicate high levels of PAHs in sediments of the impoundment

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(Entrix-Incorporated, 2000), as well as high levels of arsenic, barium, chromium, cobalt and lead (Tetra Tech, Inc, unpublished data to USEPA).

Snapping turtles (*C. serpentina*) are well-established bioindicators of environmental contamination (Albers et al., 1986; Bishop, 1990; Bishop et al., 1995; Golet and Haines, 2001; Pagano et al., 1999; Ryan et al., 1986; Stone et al., 1980). Their prevalence in wetland habitats, size, long life span, reproductive habits and large clutch size make them excellent organisms for studies of reproductive toxicity (Bergeron et al., 1994; Bishop et al., 1991, 1996; Bishop and Gendron, 1998). They feed at the top of the aquatic food chain and can live for decades, which afford them ample opportunity to accumulate biomagnified toxins. Stone et al. (1980) suggested that they may also be exposed to toxins via dermal absorption from water and sediment and absorption through mucous membranes during cloacal and pharyngeal respiration. Contaminants in painted turtles (*C. picta*) have not been as thoroughly studied as in snapping turtles (but see Meeks, 1968; Owen and Wells, 1976; Punzo et al., 1979; Reeves et al., 1977; Rie et al., 2000; Yawetz et al., 1997), but their wide geographic range, ease of capture, long life span and abundant numbers make them a viable candidate for toxicity studies.

Many of the substances found in the impoundment cause developmental abnormalities in turtles and birds (Anwer and Mehrotra, 1986; Bishop et al., 1991, 1998; Hoffman and Gay, 1981) and a prior study reports that deformities and low hatching success occur in snapping turtles from the impoundment (Steyermark, 1999). Therefore, the object of this study was to determine the extent and severity of deformities in embryos and hatchlings of turtles that lived in the refuge. We compared these data to rates of deformities in hatchlings from the E.S. George Reserve in Michigan and to other published reports of abnormalities.

2. Materials and methods

2.1. Turtle identification and egg collection

We collected snapping turtles and painted turtles by hand during the nesting season (2000–2003) or in hoop nets baited with canned sardines and canned corn during spring and summer (2000–2002). We marked every turtle captured by drilling holes or filing notches in marginal scutes following an alphabetic system similar to the numeric system used by Cagle (1939). We conducted foot patrols of nesting areas in the JHNWR in May and June, 2000–2003. When possible, we collected eggs from females as they were laid in the field, otherwise we transported gravid females to the laboratory and induced oviposition with oxytocin (Ewert and Legler, 1978; 19–21 IU/kg as per Steyermark, pers. communication). We visually examined each turtle for external deformities of limbs, shell, head and tail.

In 2000 we incubated eggs in a Rheem environmental chamber at 26.5 ± 1.0 °C. In June 2000 the incubator overheated to 40 °C and shut down. It was above 32 °C for several hours. We transferred eggs to a Precision™ incubator at 26.5 ± 0.5 °C and used this incubator for the rest of the study. Eggs were incubated in 32 cm × 18 cm plastic boxes with sand kept at 4% moisture gravimetrically. We replenished moisture in each box three times per week. Eggs that developed a white spot and continued to show signs of embryonic development were incubated full term. We dissected eggs that did not form a white spot or that showed signs of embryonic death such as arrested development (as seen by "candling"), discoloration, loss of turgidity, or foul smell. We staged snapping turtle embryos according to Yntema (1968) and painted turtle embryos according to Mahmoud et al. (1984). We examined each embryo microscopically for the same types of deformities seen in adults and any other abnormalities.

We developed a system to rate the severity of deformities seen in embryos (Table 1). All types of deformities listed in Table 1 occurred in samples taken over the four years of this study. We divided observed malformations among three categories: minor, moderate and lethal. Minor deformities were those that did not appear to affect behavior or survival of the individual in any way, because we observed them in adults in the refuge that acted normally and had normal length–mass relationships (unpublished data). Moderate deformities were those that we assumed would lower chances of survival of the individual, but which we observed in adults in the refuge. Examples of moderate deformities seen in adults included unfused maxilla or mandible bones, kyphosis, scoliosis, a single deformed leg and two deformed feet. Lethal deformities were those that would result in death due to developmental problems or that would make hatchlings unable to escape from predation. Such deformities included but were not limited to lack of eyes, brain abnormality, no carapace or plastron or more than one missing or deformed leg. Obviously we did not observe any lethal deformities in adults.

We used the E.S. George Reserve (ESGR) near Pinckney, Michigan, home to a well-studied population of snapping turtles and painted turtles (Congdon and Gibbons, 1996) as a reference site. There was no indication of contamination by PAHs or other pollutants in the water, sediments or turtles in the Reserve. We collated data on deformities in hatchling and adult snapping turtles

Table 1
System for rating severity of deformities in embryos and adults of snapping turtles and painted turtles at the JHNWR

Severity of deformity	Types of deformities
Minor: not likely to affect survival	• Misshapen carapace or plastron • Deformed tail • Extra, missing or misshapen scutes • Deformed or missing digits or 1 foot • Lack of pigmentation • Narrow body • Few or no scales
Moderate: may lower chances of survival but have seen adults with some of these conditions	• Three or more of the above minor deformities • Skull smaller than normal • One missing limb or eye • Kyphosis or scoliosis • One side of maxilla did not fuse with nasal process • Two deformed feet • Developmental Asynchrony
Lethal: probability of survival slim, no adults seen with these conditions	• Dwarf • Gastrotrichsis • Two or more limbs missing • All brain abnormalities: exencephaly, hydrocephaly, missing lobes, etc. • Missing or severely deformed jaw parts: maxillary processes not fused, maxillary process not fused with nasal process, absence of maxilla or mandible, malocclusions, etc. • Missing plastron or carapace • Ectocardia • Anophthalmia • Skull not fused • Spine/notochord bent in two or more places so that movement is impaired