HEALTH ASSESSMENT OF FREE-RANGING CHELONIANS IN AN URBAN SECTION OF THE BRONX RIVER, NEW YORK, USA

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ABSTRACT: The Bronx River in Bronx, New York, US spans an area of significant human development and has been subject to historic and ongoing industrial contamination. We evaluated the health of free-ranging native common snapping turtles (Chelydra serpentina) and nonnative invasive red-eared sliders (Trachemys scripta) in a segment of the Bronx River between May and July 2012. In 18 snapping turtles and nine sliders, complete physical examinations were performed, ectoparasites collected, and blood was analyzed for contaminants (mercury, thallium, cadmium, arsenic, lead, selenium, oxychlordane, alpha-chlordane, dieldrin, DDD, DDE, polychlorinated biphenyls). Complete blood counts and the presence of hemoparasites were determined in 16 snapping turtles and nine sliders. Swabs of the choana and cloaca were screened for ranavirus, adenovirus, herpesvirus, and Mycoplasma spp. by PCR in 39 snapping turtles and 28 sliders. Both turtle species exhibited bioaccumulation of various environmental contaminants, particularly organochlorines and polychlorinated biphenyls. Molecular screening revealed a unique herpesvirus in each species. A Mycoplasma sp. previously isolated from emydid turtles was detected in red-eared sliders while a unique Mycoplasma sp. was identified in common snapping turtles. Ranaviruses and adenoviruses were not detected. Our study established a baseline health assessment to which future data can be compared. Moreover, it served to expand the knowledge and patterns of health markers, environmental contaminants, and microorganisms of free-ranging chelonians.

Key words: Chelonians, environmental contaminants, herpesvirus, infectious disease, mycoplasma, red-eared slider, snapping turtle, turtles.

INTRODUCTION

The Bronx River in New York State, US, a site of historic and ongoing industrial contamination, is the focus of a collaborative effort led by the National Oceanic and Atmospheric Administration and the Wildlife Conservation Society to restore river habitat through a variety of projects including community education, reintroducing native fish species, and wildlife health monitoring. The Bronx River originates in northern Westchester County, New York, courses southward into the Bronx, and terminates as a minor tributary to New York Harbor. The north section of the river, extending from Westchester County to the New York Botanical Garden (NYBG), has been previously channelized and possesses little suitable habitat for chelonians. The middle section, coursing through the NYBG and the Bronx Zoo (BZ), has been designated parkland since the 1800s and contains a large amount of suitable habitat for turtles. The south section, extending from the border of the BZ to the East River, is industrialized, brackish, and is generally poor habitat for chelonians (Litten 2003).

The impact of environmental contaminants on aquatic animal health is poorly understood, particularly for chelonians. Turtles are effec-
tive biomonitors for environmental contamination, as they absorb contaminants through various routes including ingestion and skin, accumulate large amounts of body fat, and occupy high trophic levels within their ecosystems (Golden and Rattner 2003).

Common snapping turtles (Chelydra serpentina) have a large geographic distribution spanning the eastern two thirds of North America from southern Canada to southern Texas (Ernst 2008). Their nonmigratory habits and longevity make them an ideal species for assessing the effects of environmental contamination. Red-eared sliders (Trachemys scripta) are naturally found south of Virginia to Venezuela and are invasive, nonnative species introduced to many parts of the US including parts of the Northeast (Ernst 1990). The main purpose of this study was to develop a baseline health assessment for common snapping turtles and red-eared sliders in the Bronx River.

MATERIALS AND METHODS

Sampling location and collection

We trapped animals from May 2012 through July 2012 in the NYBG and BZ section of the river using 0.9- and 1.2-m–diameter hoop traps and basking traps (Memphis Net and Twine, Memphis, Tennessee, USA). We captured several snapping turtles by hand in shallow waters. We manually restrained turtles, measured carapace length and width, and determined sex by evaluating external sexually dimorphic features (Boyer and Boyer 2006). We collected a combined choanal and cloacal swab (BD CultureSwab™, Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA) from each turtle and stored swabs dry, up to 8 h, until transfer to the laboratory where they were stored frozen (–80 C) until pathogen analysis was performed (310–371 d later). We identified ectoparasites to the species level by morphologic analysis using taxonomic keys (Klemm 1982, 1985; Moser et al. 2016).

We analyzed heavy metals, organochlorines (OCs), polychlorinated biphenyls (PCBs), and mercury levels in a subset of animals at the Pennsylvania Animal Diagnostic Laboratory System, New Bolton Center Toxicology Laboratory, University of Pennsylvania, School of Veterinary Medicine (Kennett Square, Pennsylvania, USA). Mercury in whole blood was analyzed using an atomic absorption spectrometer AA800 equipped with FIAS 400 and AS-90 autosampler (PerkinElmer, Shelton, Connecticut, USA) by cold vapor atomic absorption. A reagent blank, calibration blank, four working calibration standards, and a certified reference material (DOLT-3) from the National Research Council Canada (Ottawa, Ontario, Canada) were incorporated with each run. The mercury method detection limit was established at 0.3 µg/g.

Inductively coupled plasma-mass spectrometry NexION 300D ICP-MS equipped with S-10 autosampler (PerkinElmer) was used for arsenic, cadmium, lead, thallium, and selenium analysis in whole blood. A reagent blank, calibration blank, and four working calibration standards were incorporated, and the performance of the ICP-MS and the accuracy of the results were monitored by analyzing Standard Reference Material 1577c from the National Institute of Standards and Technology (Gaithersburg, Maryland, USA) with each run. Method detection limits were established as follows: 0.001 µg/g for cadmium and thallium; 0.01 µg/g for lead; 0.05 µg/g for arsenic and selenium.

The Monsanto Company (Creve Coeur, Missouri, USA) previously manufactured unique mixtures of PCBs (e.g., Aroclor 1260) under the now-expired Aroclor trademark (Litten 2003). Organochlorines and PCBs in plasma were analyzed on a gas chromatograph (Agilent 6890, Agilent, Santa Clara, California, USA) equipped with dual electron capture detectors using two columns, Restek 50 (Bellefonte, Pennsylvania, USA) for quantification and J&W Scientific HP 5 MS (Agilent) for confirmation. A calibration curve using tetrachloro-m-xylene as the internal standard was prepared with serum spiked with

Sample analysis

We measured packed cell volume percentage (PCV%) using centrifuged hematocrit tubes and plasma total protein by refractometer. Total white blood cell (WBC) counts (estimated) and differential WBC counts were performed within 7 d of collection, all by the same investigator (K.I.). Aliquots of whole blood and plasma prepared by centrifugation were stored frozen (–80 C) until toxicoanalysis was performed (up to 197 d later). We identified ectoparasites to the species level by morphologic analysis using taxonomic keys (Klemm 1982, 1985; Moser et al. 2016).

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standards for OCs from 5–100 ng/g and PCBs from 50–500 ng/g. The method detection limits for the following compounds were: 5 ng/g for pp-DDE, pp-DDD, dieldrin, oxychlordane, a-chlordane; 100 ng/g for Aroclor 1260.

We performed nucleic acid extraction from combined choanal and cloacal swabs from 67 turtles (39 snapping turtles and 28 sliders) using a QIAamp DNA Mini Kit (Qiagen Inc., Valencia, California, USA) per the manufacturer’s recommendations. Samples were screened for the ranavirus major capsid protein by quantitative PCR using a consensus primer and probe set as previously described (Pallister et al. 2007). Qualitative nested PCR amplification of 181 base pairs (bp) and 689-bp fragments of the herpserviral DNA-dependent DNA polymerase were performed using previously described methods (VandeVanter et al. 1996; Ossiboff et al. 2015a). For Mycoplasma and adenovirus testing, qualitative PCR amplification of an approximately 500-bp or 330-bp segment of the Mycoplasma 16S-23S bacterial intergenic spacer (IGS) region or adenoviral DNA polymerase gene, respectively, was performed (Wellehan et al. 2004; Ossiboff et al. 2015b).

For all qualitative PCR reactions, we visualized amplified products with gel electrophoresis and we submitted amplicons of the expected size for direct Sanger sequencing (Genewiz, Inc., South Plainfield, New Jersey, USA). We edited, trimmed, and aligned the DNA sequences using Geneious bioinformatics software (version 6.1.7; Biomatters, Ltd., Auckland, New Zealand). We performed sequence analysis using BLAST in GenBank (National Center for Biotechnology Information 2017), and uploaded sequences to GenBank (accession nos. MG677112–MG677115).

We performed Bayesian phylogenetic analysis on deduced amino acid sequences of the 689-bp fragment of the partial herpesvirus DNA polymerase gene using Geneious bioinformatics software (version 10.1.3) and MrBayes plugin for Geneious as previously described (Huelsenbeck and Ronquist 2001; Ossiboff et al. 2015a). We visualized phylogenetic trees using FigTree software (version v1.4.3, Rambaut 2016).

RESULTS

We captured a total of 69 turtles (41 common snapping turtles and 28 red-eared sliders). There were 13 male, 20 female, and eight undetermined sex snapping turtles and nine male, 18 female, and one undetermined sex sliders. Weights ranged from 0.136–2.55 kg (n=17) in sliders and 0.709–14.4 kg (n=29) in snapping turtles.

Physical examinations

Eighteen common snapping turtles received a complete physical exam. Two snapping turtles had deformed or flattened rostrums and one had clubbing of the left thoracic limb and distal tail. Evidence of previous trauma (e.g., carapacial scars, healing wounds on limbs) was noted in 28% (5/18) of snapping turtles. Complete physical exam of nine red-eared sliders revealed no abnormalities.

Leeches were found in 72% (13/18) of common snapping turtles and 56% (5/9) of red-eared sliders. All leeches were identified as various stages (hatchling, juvenile, or adult) of Placobdella parasitica. No lesions were associated with the presence of the leeches.

Hematology and environmental contaminant analysis

A summary of results of the hematology analyses is provided in Table 1. Common snapping turtles exhibited a greater range in total WBCs compared to red-eared sliders, and the total WBC mean in snapping turtles was greater than the median in red-eared sliders. Differential WBC counts revealed higher relative heterophil counts in red-eared sliders, higher relative basophil counts in common snapping turtles, and similar relative counts in lymphocytes, eosinophils, monocytes, and azurophils. The mean values and range of PCV% were similar among species;
Table 1. Hematology values for nine free-ranging red-eared slider turtles (Trachemys scripta) and 16 free-ranging common snapping turtles (Chelydra serpentina) captured between May and July 2012 from the Bronx River, Bronx, New York, USA. Means (SD) are reported for normally distributed data and medians are reported for nonnormally distributed data. Dashes (—) indicate data is not applicable.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Red-eared sliders</th>
<th>Common snapping turtles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Packed cell volume (%)</td>
<td>19.3</td>
<td>5.3</td>
</tr>
<tr>
<td>Total solids (g/dL)</td>
<td>2.3</td>
<td>1.5</td>
</tr>
<tr>
<td>Total white blood cells (×1,000/μL)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Heterophils (×1,000/μL)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Lymphocytes (×1,000/μL)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Eosinophils (×1,000/μL)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Monocytes (×1,000/μL)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Azurophils (×1,000/μL)</td>
<td>—</td>
<td>0.098</td>
</tr>
<tr>
<td>Basophils (×1,000/μL)</td>
<td>2.1</td>
<td>2.4</td>
</tr>
<tr>
<td>Heterophils relative (%)</td>
<td>27</td>
<td>17</td>
</tr>
<tr>
<td>Lymphocytes relative (%)</td>
<td>43</td>
<td>11</td>
</tr>
<tr>
<td>Eosinophils relative (%)</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>Monocytes relative (%)</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>Azurophils relative (%)</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>Basophils relative (%)</td>
<td>—</td>
<td>9</td>
</tr>
</tbody>
</table>

However, the range was slightly greater in red-eared sliders. Hematology reference values have not been previously published for common snapping turtles. However, PCV% in common snapping turtles with similar blood lead levels have been reported (18.54%, Overmann and Krajicek 1995), and the mean PCV% in snapping turtles (22%) in this study was slightly higher. Compared to previously reported hematology values in red-eared sliders (Diethelm and Stein 2006), the red-eared sliders in this study exhibited a similar range in total WBC, lower total WBCs, and lower mean PCV%. Hemoparasites (Hemogregarina sp.) were detected in 75% (12/16) of common snapping turtles and 33% (3/9) of red-eared sliders.

A summary of results of the blood concentration of contaminants is provided in Table 2. Mercury levels were below the level of detection (≥0.3 μg/g) in whole blood samples from eight common snapping turtles and five red-eared sliders. However, a variety of heavy metals was detected in whole blood samples from 10 snapping turtles and four sliders. Thallium and cadmium were not detected in snapping turtles, but were both detected in the same female red-eared slider. No significant physical exam abnormalities were noted in this individual; however, it exhibited a high relative heterophil count (47%) and generally lower PCV% and total solids (16%, 1.0 g/dL).

We analyzed plasma samples from 26 turtles (17 snapping turtles and nine sliders) for the presence of OCs (oxychlordane, alphachlordane, dieldrin, DDD, and DDE) and PCBs (Aroclor 1260). We detected OCs in variable amounts. We detected oxychlordane in all common snapping turtles over a large range (6.5–96 ng/g) and in approximately half of sampled red-eared sliders over a smaller range (6.8–16.8 ng/g). We detected alphachlordane in 47% (8/17) of common snapping turtles and 56% (5/9) of red-eared sliders over...
Table 2. Concentrations of contaminants in whole blood (WB) or blood plasma (PL) from red-eared sliders (*Trachemys scripta*) and common snapping turtles (*Chelydra serpentina*) captured between May and July 2012 from the Bronx River, Bronx, New York, USA. Mean (SD) is reported for normally distributed data and median is reported for nonnormally distributed data. Ranges exclude animals in which contaminants were not detected. Dashes (—) indicate data is not applicable.

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Sample type</th>
<th>Detection limit</th>
<th>n</th>
<th>% Detected</th>
<th>Range</th>
<th>Mean (SD)</th>
<th>n</th>
<th>% Detected</th>
<th>Range</th>
<th>Mean (SD) or median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mercury (μg/g)</td>
<td>WB</td>
<td>0.3</td>
<td>5</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>8</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Thallium (μg/g)</td>
<td>WB</td>
<td>0.001</td>
<td>4</td>
<td>25</td>
<td>0.005</td>
<td>—</td>
<td>10</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cadmium (μg/g)</td>
<td>WB</td>
<td>0.001</td>
<td>4</td>
<td>25</td>
<td>0.005</td>
<td>—</td>
<td>10</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Arsenic (μg/g)</td>
<td>WB</td>
<td>0.05</td>
<td>4</td>
<td>25</td>
<td>0.063</td>
<td>—</td>
<td>10</td>
<td>30</td>
<td>0.071–0.15</td>
<td>—</td>
</tr>
<tr>
<td>Lead (μg/g)</td>
<td>WB</td>
<td>0.01</td>
<td>4</td>
<td>100</td>
<td>0.054–0.137</td>
<td>0.091 (0.034)</td>
<td>10</td>
<td>100</td>
<td>0.090–0.576</td>
<td>0.33 (0.15)</td>
</tr>
<tr>
<td>Selenium (μg/g)</td>
<td>WB</td>
<td>0.05</td>
<td>4</td>
<td>100</td>
<td>0.114–0.290</td>
<td>0.206 (0.072)</td>
<td>10</td>
<td>100</td>
<td>0.103–0.540</td>
<td>0.366 (0.121)</td>
</tr>
<tr>
<td>Oxychlordane (ng/g)</td>
<td>PL</td>
<td>5</td>
<td>9</td>
<td>56</td>
<td>6.8–16.8</td>
<td>—</td>
<td>17</td>
<td>100</td>
<td>6.5–96</td>
<td>23</td>
</tr>
<tr>
<td>Alpha-chlordane (ng/g)</td>
<td>PL</td>
<td>5</td>
<td>9</td>
<td>56</td>
<td>7.8–9.2</td>
<td>—</td>
<td>17</td>
<td>47</td>
<td>5.6–10.8</td>
<td>—</td>
</tr>
<tr>
<td>Dieldrin (ng/g)</td>
<td>PL</td>
<td>5</td>
<td>9</td>
<td>33</td>
<td>5.4–9.4</td>
<td>—</td>
<td>17</td>
<td>53</td>
<td>5.1–11.1</td>
<td>—</td>
</tr>
<tr>
<td>DDD (ng/g)</td>
<td>PL</td>
<td>5</td>
<td>9</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>17</td>
<td>18</td>
<td>5–7</td>
<td>—</td>
</tr>
<tr>
<td>DDE (ng/g)</td>
<td>PL</td>
<td>5</td>
<td>9</td>
<td>56</td>
<td>5.1–34.7</td>
<td>—</td>
<td>17</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Aroclor 1260 (ng/g)</td>
<td>PL</td>
<td>100</td>
<td>9</td>
<td>44</td>
<td>101–408</td>
<td>—</td>
<td>17</td>
<td>59</td>
<td>106–246</td>
<td>—</td>
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</table>
similar ranges. We detected dieldrin in both species that was also over similar concentration ranges. We detected DDD in a low number of common snapping turtles (3/17) and not in any red-eared sliders. In contrast, we did not detect DDE in any common snapping turtles but found it in 56% (5/9) of red-eared sliders.

We detected polychlorinated biphenyls (Aroclor 1260) in nearly 60% of common snapping turtles (range: 106–246 ng/g) and nearly 50% of red-eared sliders (range: 101–408 ng/g). One slider had a notably higher (408 ng/g) level than did the eight other individuals sampled (101–134 ng/g). There were no significant physical exam findings in this individual; however, this female exhibited the lowest PCV% and total solids (11%, 0.5 g/dL) among sampled individuals.

Detection of mycoplasmas and viruses

We did not detect ranavirus and adenovirus in any swabs. Swab extracts were positive for herpesvirus in 26% (10/39) of common snapping turtles and 11% (3/28) of red-eared sliders; the prevalence between the species was not significantly different ($P=0.210$). The herpesviral sequences amplified from the three red-eared sliders were identical to each other and 99.7% similar to an isolate previously reported from a red-eared slider (Sim et al. 2015). Other sequences with the highest similarity to the red-eared slider herpesvirus that we detected, Trachemys herpesvirus 1 (GenBank no. MG677114), included other fresh water turtle herpesviruses: Emydid herpesvirus 1 (GenBank no. KM357866; 82%), Glyptemys herpesvirus 1 (GenBank no. KM357867; 79%), and Emydid herpesvirus 2 (GenBank no. KM357868; 78%). The herpesviral sequences amplified from snapping turtle samples were 99.5–100% identical to each other but only 76.2–76.8% identical to Trachemys herpesvirus 1. The snapping turtle herpesvirus we detected, Chelydra herpesvirus 1 (GenBank no. MG677113), was 89% identical to Glyptemys herpesvirus 1 (GenBank no. KM357867), 88% to Emydid herpesvirus 2 (GenBank no. KM357868), and 85% to Glyptemys herpesvirus 2 (GenBank no. KM357869). Phylogenetic analysis placed both herpesviruses in a single clade with all other reported chelonid herpesviruses, including Chelonid herpesvirus 5, the type virus of the genus Scutavirus (Fig. 1).

Mycoplasma spp. were detected in 36% (14/39) of common snapping turtles and 7% (2/28) of red-eared sliders, a significant difference ($P=0.008$). The DNA sequence from the 479-bp 16S-23S IGS region (GenBank no. MG677114) was identical in both sliders and was 99–100% identical to a Mycoplasma sp. previously reported in wild emydid turtles of the northeastern US and in a captive ornate box turtle (Terrapene ornata; Farkas and Gál 2009; Ossiboff et al. 2015b), 89% identical to Mycoplasma agassizii, 78% identical to Mycoplasma testudineum, and 73% identical to Mycoplasma pulmonis. The DNA sequences from the 455-bp 16S-23S IGS region of 11 snapping turtles (GenBank no. MG677115) were retrieved from four animals due to sequencing challenges; however, all partial sequences were 100% identical to sequences from the other 11 snapping turtles.

DISCUSSION

The majority of turtles was grossly normal on physical exam; however, the few abnormalities noted may be the result of previous trauma or congenital deformity. Hemoparasites (Hemogregarina sp.) and leeches (P. parasitica) were seen in both turtle species. This leech species is known to parasitize snapping turtles in other systems (Brooks et al. 1990), and Placobdella spp. can transmit hemogregarine parasites along a broad host range (Siddall and Desser 2001). Leeches and hemoparasites were found in a greater proportion of common snapping turtles than in
red-eared sliders in the Bronx River, and further investigation is needed to determine the role of leeches in the transmission of hemoparasites in this system.

Packed cell volume percent and hemoglobin (Hb) concentration reflect red blood cell content in whole blood, and long-term exposure to lead in slider turtles has been associated with a 10% decline in Hb (Lovelette and Wright 1996). Lead was detected in all common snapping turtles and red-eared sliders sampled, and common snapping turtles exhibited a higher mean PCV% compared to red-eared sliders despite higher levels of lead and hemoparasites. Hematology results were generally consistent with ranges reported in other chelonians (Diethelm and Stein 2006); however, interpreting the differences in PCV%, total WBCs, and relative WBC counts in Bronx River turtles is challenging due to the many factors that influence hematology results including site of sample collection (López-Olvera et al. 2003), geographic location (Chaffin et al. 2008), sex, season (Chung et al. 2009), and captivity status (Keller et al. 2012).

Molecular screening for pathogens identified Mycoplasma spp. and herpesviruses in both turtle species. We detected a Mycoplasma sp. in a small subset of the red-eared sliders that was 99–100% identical to one previously reported in other wild, emydid turtles of the northeastern US including bog (Glyptemys muhlenbergii), spotted (Clemmys guttata), eastern box (Terrapene carolina carolina), and wood turtles (Glyptemys insculpta; Ossiboff et al. 2015b). While this Mycoplasma sp. is suspected to be a commensal organism in several wild emydid species, it has been associated with upper respiratory disease in wild eastern box turtles (T. c. carolina; Palmer et al. 2016). The significance of this Mycoplasma sp. in the red-eared sliders is unclear. The snapping turtle Mycoplasma sp. had a 16S-23S IGS sequence unique to both that of the Mycoplasma sp. detected in the red-eared sliders as well as previously reported Mycoplasma spp.,
and possibly represents a novel *Mycoplasma* species. Complete 16S-23S IGS sequence of the organism was determined in 11 of the PCR-positive snapping turtles. In three additional turtles, only partial sequence determination was possible due to regions of overlapping signal following areas of clear electropherogram peaks, raising the possibility of the presence of a mixture of *Mycoplasma* spp. in those individuals.

We also detected herpesvirus in both of the species studied. Trachemys herpesvirus 1, detected in 3/28 red-eared sliders, was 99.7% identical to a herpesvirus previously detected in a red-eared slider (Sim et al. 2015). The Chelydra herpesvirus 1 detected in common snapping turtles was unique, not similar to previously reported chelonian herpesvirus, and likely represents a novel chelonian herpesvirus. Phylogenetic analysis placed both herpesviruses in close relation to other herpesviruses of freshwater turtles, and deep within the clade containing all other chelonian herpesviruses, including Chelonid Herpesvirus 5, the type virus of the genus *Scutavirus*. The significance of these viruses to the health of the study population is unclear. Herpesviruses have been regularly detected in health assessments of overtly healthy wild turtle populations (Marschang et al. 2015; Ossiboff et al. 2015a; Kane et al. 2016), but they have also been associated with disease and mortality in freshwater turtles (Sim et al. 2015).

Contaminants enter the environment through various sources, including the use of insecticides and preservatives and through industrial effluents. After an animal is exposed, toxins circulate readily through blood, but generally concentrate in specific tissue types: OCs and PCBs in hepatic and adipose tissue; metals in hepatic, renal, and variably in other tissues. An exception is lead, which circulates for extended periods in erythrocytes but is stored in bone long-term (Thompson 2018). Obtaining organ tissue samples as part of a capture and release study presents technical and ethical challenges beyond the scope of this study. Although measuring blood concentrations likely underestimates total body burden for many contaminants, blood collection was pursued as a relatively noninvasive and safe sampling method.

The manufacture of PCBs for various industrial uses ceased in 1977 due to concerns regarding their detrimental impact on human health. They have been shown to influence embryonic development in chelonians (Khan and Thomas 1997), in particular causing developmental deformities in snapping turtles (Bishop et al. 1998) and male-to-female sex shifts in red-eared slider eggs (Bergeron et al. 1994). Water quality analysis of the Bronx River near the NYBG conducted by the US Geological Survey showed that in August 2012, there were no detectable PCBs (detection limit 0.1 µg/L=0.1 ng/g; NWQMC 2018). Previous Bronx River water quality analysis near the NYBG and BZ performed between 1998 and 1999 by the New York Department of Environmental Conservation showed the average sum of all PCB homologues was 4.52 ng/L (0.0045 ng/g; Litten 2003). Comparing these findings to turtle blood PCB concentrations illustrates a pattern of bioaccumulation. Additionally, PCB levels in Bronx River turtles are similar to plasma concentrations seen in turtles from other contaminated sites (263–415 ng/g; de Solla et al. 1998) and are higher than levels found in turtles from pristine or low-contaminated sites (10–18 ng/g; Moss et al. 2009).

Organochlorines also impact a variety of body systems and can inhibit adenosine triphosphatase formation in various chelonian body tissues (Phillips and Wells 1974). Various abnormal health conditions associated with OC exposure have been noted in chelonians including conjunctivitis, otitis media (Tangredi and Evans 1997), aural abscesses (Holladay et al. 2001), lower lysozyme activity (a marker of innate immunity; Keller et al. 2006), and abnormal sexual development (Willingham and Crews 1999). Comparing OC levels from this study to Bronx River waters near the NYBG in 2012 (dieldrin=0.01 ng/g; chlorodanes, DDD, DDE were not detected; NWQMC 2018) illustrates a pattern of bioaccumulation for all OCs tested. In addition, dieldrin and alpha-chlordane levels in this study were higher compared to plasma...
levels in common snapping turtles from contaminated sites in Ontario, Canada that exhibited feminization of genital morphology (de Solla et al. 1998). Genital morphology was not measured in the current study; however, the impact of OCs on sexual development in Bronx River turtles warrants further research.

Mercury was not detected in any turtles sampled despite an average river water concentration of dissolved mercury of 0.063 ng/g (NWQMC 2018). In contrast, mercury was detected in alligator snapping turtles (Macrochelys temminckii) in Florida and Georgia (Chaffin et al. 2008) and red-eared sliders in Texas (Clark et al. 2000) using a lower detection limit of 0.01 μg/g. Thus, a lower detection limit would likely improve future analyses in Bronx River turtle populations.

All turtles sampled in this study tested positive for lead, and the river water concentration of lead (0.0421 μg/g; NWQMC 2018) was slightly lower than in blood levels detected in this study. Lead exposure in common snapping turtles and red-eared sliders has been correlated with decreased activity of a synthetic enzyme (aminolevulinic acid dehydratase) of heme in erythrocytes and has been associated with a 10% decrease in Hb in the latter species (Lovelette and Wright 1996). Burger et al. (1998) also linked lead exposure to prolonged righting reflex times in red-eared slider hatchlings, which can indirectly impact survivorship. In addition, cadmium, selenium, arsenic, and thallium exposure is associated with impaired reproduction (Kitana and Callard 2008), decreased metabolic rates (Nagle et al. 2001), and toxic effects on the gastrointestinal tract and neurologic system (Garland 2018; Hooser 2018). Although mercury, thallium, cadmium, and arsenic were detected in a low number of animals tested, tissue sampling of liver and kidney would represent more-accurate levels of whole body contamination.

A major limitation of this study was a small sample size. In addition, mercury and heavy metal analysis was not completed in some individuals due to limited volumes of blood samples collected. Thus, the lack of complete contaminant analysis for every animal that was physically examined, sampled for hematologic analysis, and sampled for microorganism testing limits our ability to assess potential relationships between these different markers. In addition, some of the assay detection limits and the use of blood instead of organ tissue for analysis may have limited our ability to detect contaminants within these animals. Still, this study identified high levels of multiple toxins (e.g., OCs, PCBs, lead, selenium, Mycoplasma spp.) that warrant more-detailed study in the future.

Despite several limitations, the results of this study establish baseline data regarding the health status, presence of pathogens, and the levels of environmental toxins in chelonians in the Bronx River. While previous chelonian studies provide some context for this data, different populations even of the same species exhibit natural variation and local adaptation. Turtle populations in the Bronx River will continue to face ecosystem change, particularly due to restoration projects but also in response to stochastic events, introduction of nonnative species, and natural environmental change. This study provides the basis for comparison for future studies in this river system as conditions change and also to other river systems.

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LITERATURE CITED


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