SEXUALLY DIMORPHIC MORPHOLOGY OF HATCHLING SNAPPING TURTLES (CHELYDRA SERPENTINA) FROM CONTAMINATED AND REFERENCE SITES IN THE GREAT LAKES AND ST. LAWRENCE RIVER BASIN, NORTH AMERICA

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Abstract—Some organochlorine pesticides and industrial chemicals may alter sexually dimorphic traits through endocrine disruption. Therefore, we examined a sexually dimorphic trait, precloacal length, of hatching snapping turtles (Chelydra serpentina) incubated from 31 clutches from a heavily contaminated site (Hamilton Harbour, ON, Canada; n = 14), a moderately contaminated site (Akwesasne Mohawk Territory; n = 3), and from a reference site (Algonquin Provincial Park, ON, Canada; n = 14). The mean sum polychlorinated biphenyls from Algonquin were low (20.33 ng/g wet wt), but were high from both Hamilton and Akwesasne (2,956.28 and 3,377.0 ng/g wet wt, respectively). Organochlorine pesticides, such as dichlorodiphenyldichloroethylene, were low from Algonquin (1.67 ng/g wet wt), moderate from Akwesasne (10.00 ng/g wet wt), and relatively high from Hamilton (135.14 ng/g wet wt). At all sites, the precloacal length of male hatching was larger than that of females by an equal amount at any given body size. However, the precloacal length of both males and females from Hamilton increased with body size at a slower rate than of males and females from the other two sites. Our results support an earlier study that found differences in sexually dimorphic morphology of adult snapping turtles among contaminated and uncontaminated sites. Furthermore, these alterations in secondary sexual characteristics previously observed in adults likely are initiated early in development, and may result in permanent organizational changes in morphology.

Keywords—Chlorinated hydrocarbons Morphology Sex Sexual dimorphism Snapping turtles

INTRODUCTION

The classic organizational theory of the development of secondary sexual characteristics states that sex hormones affect the development of sexually dimorphic traits early in life through permanent organizational effects [1]. Organizational effects usually occur during a critical period of development and are hypothesized to be important for traits that have developmentally fixed alternatives [2]. The activational theory, on the other hand, states that sex hormones temporarily activate sexually dimorphic traits in adults [1], and may be important for traits that have developmentally plastic alternatives [2]. Hormone analogs such as diethylstilbestrol can bind to hormone receptors and thereby affect sexually dimorphic traits in a manner similar to, or even more strongly than, natural hormones [3,4]. Organochlorine pesticides and industrial chemicals also may alter sexually dimorphic traits through organizational pathways [5,6].

Many organochlorine compounds will competitively bind to estrogen or androgen receptors [7,8], and alter gene expression of sex hormone–linked traits [8–10]. Laboratory studies have confirmed that sexually dimorphic morphology in mammals can be affected by some organochlorine compounds, such as p,p′-dichlorodiphenyldichloroethylene (p,p′-DDE), 2,3,7,8-tetrachlorodibenzop-12-dioxin, polychlorinated biphenyls (PCBs), and dibenzo furans [7,11,12]. Correlations between environmental exposure to endocrine-disrupting compounds and altered secondary sexual characteristics also have been found in free-ranging mammals and fish [13,14]. Many sexually dimorphic traits in reptiles, such as brain morphology [15], behavior [15,16], coloration [17], and body size [17], are affected by sex hormone levels, and thus may be sensitive to endocrine-disrupting compounds. Although few studies have examined effects of organochlorines on reptiles, evidence exists that endocrine disruptors affect secondary sexual characteristics in both alligators (Alligator mississippiensi) and snapping turtles (Chelydra serpentina) [18,19].

Snapping turtles from contaminated sites in the Great Lakes area in Ontario, Canada, have high levels of organochlorine compounds, primarily PCBs and pesticides [20], and females can transfer potentially hazardous levels of organochlorines to their offspring. Previous work indicates that offspring that are exposed to maternally transferred contaminants have an increased incidence of developmental malformations [20]. Evidence also exists that sexually dimorphic traits in adult snapping turtles differed among contaminated and reference sites [19]. The precloacal length, which is the distance between the posterior end of the plastron and the cloaca (Fig. 1), is a secondary sexual characteristic in turtles and is typically longer in adult males compared to adult females [21]. Because this is where the penis is located. The precloacal area grows faster than the carapace in males, but not in females [22]. In both sexes, the precloacal length was shorter in adults from contaminated Great Lake sites compared to reference sites [19].

The differences in the precloacal length of adult snapping
temperatures between 22 and 28°C, which males are produced when eggs are incubated at constant 25°C. Snap- ping turtles have temperature-dependent sex determination, in which males are produced outside this range. The critical period of sex determination occurs from stages 14 to 21, during the middle third period of incubation [23]. During or after the period of sex determination, differences may occur between males and females in the growth trajectory of the precloacal area. We tested whether the previously observed differences in sexual morphology of adult snapping turtles [19] were due to changes occurring during embryonic development. Snapping turtle eggs were collected from a reference site (Algonquin Provincial Park) and two contaminated sites (Hamilton Harbour, ON, Canada, and Akwesasne Mohawk Territory). Subsamples from each clutch were analyzed for organochlorine pesticides and PCBs. The remaining eggs were artificially incubated at male- and female-producing temperatures. The relative size of the precloacal area was compared between male and female hatchlings within sites and between hatchlings of the same sex from contaminated and reference sites.

Another indicator of environmental stress is fluctuating asymmetry [24]. Because we previously found that organochlorines can induce developmental malformations in the costal scutes of snapping turtles, we also measured fluctuating asymmetry of hatchlings. Fluctuating asymmetry is a result of errors in the development of a bilateral trait, resulting in slight differences in morphology between the left and right trait [25]. If chemical contaminants are disrupting normal development, then this disruption may increase the degree of fluctuating asymmetry. Therefore, the length of a costal scute on the left and right sides of the carapace of male hatchlings (Fig. 1) was measured to determine if the degree of fluctuating asymmetry varied between a contaminated and reference site.

**MATERIALS AND METHODS**

**Study areas**

Snapping turtle eggs were collected from three sites in southern Ontario, Canada, for artificial incubation in June 1998. Coote’s Paradise, Hamilton, Ontario (43°17’N, 79°53’W), is a 90-ha eutrophic wetland at the western end of Hamilton Harbour, and is adjacent to heavy industry, and is downstream from urban and agricultural land and a tertiary sewage treatment plant. The Snye Marsh of the Territory of the Mohawk Nation, Akwesasne (45°00’N, 74°35’W), is exposed to chlorinated hydrocarbons from several large, industrial landfill sites adjacent to the St. Lawrence River. Algonquin Provincial Park (45°35’N, 78°30’W) is a selectively logged parkland that contains many marshes and dystrophic lakes. All eggs collected from this site were from snapping turtles from dystrophic lakes. Algonquin Park has no history of heavy industry or agriculture. Egg samples were collected from nests within 24 h of oviposition. A subsample of five eggs was taken from most clutches for chemical analysis within 24 h of collection. The contents of the five eggs were pooled and placed in pretreated glass jars (rinsed with hexane) with a lid lined with hexane-rinsed foil and stored at −20°C until analyzed.

Fourteen clutches from Algonquin, 14 clutches from Hamilton, and three from Akwesasne were incubated, and the number of eggs per clutch incubated ranged from 20 to 47. Eggs were incubated in vermiculite, with a mass ratio of 1:1:1.0 water:vermiculite (mass/mass). All eggs were placed in cool conditions (−20°C) until placed in constant temperature rooms three weeks after collection, and a mass ratio of 1:1:1.0 water:vermiculite (mass/mass) was maintained throughout the incubation period at the Hagen Aqualab, University of Guelph (Guelph, ON, Canada). Half of the eggs were incubated at a constant 25.0°C to produce males, whereas the rest were incubated at 29.5°C to produce females. Abnormal fluctuations in temperature control in the 29.5°C room led to high mortality and thus reduced the sample size. Thus, caution should be used when interpreting the results involving the female hatchlings. Temperature fluctuations were otherwise usually within 0.5°C, and always within 1°C.

After hatching, turtles were placed into containers containing water, and approximately three months later they were weighed, measured, and euthanized. The sex of the hatchlings was identified by examining gonadal development with a dissecting microscope. Gonads were classified by shape and length, with long, oval gonads and the presence of an oviduct being characteristic of females and short, round gonads being characteristic of males [26].

**Body size and precloacal length**

To test for differences in the precloacal length, t-test were used to determine if the elevations or the slopes of the rela-
tion between carapace length and precloacal length varied among sites or among sexes [27]. Alterations in sexually dimorphic morphology can occur if the precloacal length increased at differing rates relative to body size (i.e., the regressions between precloacal and carapace length have different slopes), or if the isometric relation between precloacal length and body size differs (i.e., the regressions between precloacal and carapace length have different elevations).

**Fluctuating asymmetry**

The degree of asymmetry was estimated by the difference between the measurements on the left and right side of the hatching. The lengths of two scutes were measured, one on each side of the carapace. The measurements were taken between the junction of the first and second costal scutes and the second vertebral scute, and the intersection between the second and third vertebral scutes and the second costal scute. These measurements were taken over a 5-d period approximately 2.5 months after the first day of hatching. Before taking the measurements, the turtles were dried slightly with a paper towel to make the scutal intersections more apparent. The measurements were taken on both sides of the hatching, with the calipers held in the same relative position to the scutes, to reduce the likelihood of measurement bias. Vernier calipers were used, and the scutes were measured with a precision of 0.002 cm.

Bias in the fluctuating asymmetry measurements was estimated by testing for any differences between the mean scute length on the left and right sides from use of a paired t test. Significant differences between the mean left and right measurements would indicate a relative bias in the left and right measurements. The fluctuating asymmetry measurements were highly skewed, so nonparametric tests were used whenever possible, A Mann–Whitney U test, which compares mean ranks between treatments, and a Kolmogorov–Smirnov two-sample test, which compares the shape of the distributions (variance, kurtosis, and skewness) between treatments, were used to compare asymmetry of hatchlings between sites. The sample sizes used were 118 male hatchlings from Algonquin and 183 male hatchlings from Hamilton. Because alterations in fluctuating asymmetry tend to be small, large sample sizes are required for adequate power. Therefore, no hatchlings from Akwesasne were used because of the small sample size from that site.

**Chlorinated hydrocarbon analysis**

The egg samples were thawed to room temperature and extracted with dichloromethane:hexane (1:1, v/v) after the samples were dehydrated with anhydrous Na2SO4. The lipids and biogenic material were removed by means of gel permeation chromatography, and were further cleaned by Florisil column chromatography (Floridin, Berkeley Springs, WV, USA). The organochlorine pesticides analyzed were p,p’-DDE and related isomers (p,p’-dichlorodiphenyldichloroethane and p,p’-DDT); 1245- and 1234-tetrachlorobenzenes; pentachlorobenzene (QCB); hexachlorobenzene (HCB); octachlorostyrene (OCS); photomirex and mirex; dieldrin, trans-, cis-, and oxy- chlordane; cis- and trans-nonachlor; heptachlor epoxide; and α- and β-hexachlorocyclohexane. Fifty-nine standard PCB congeners were measured. The sum PCBs analyzed were of the following congeners: 13/29, 17, 18, 22, 28, 31, 33/20, 42, 44, 47, 49, 52, 56/60, 64, 66, 70/76, 74, 85, 87, 92, 95, 97, 99, 101/90, 105, 110, 118, 128, 130, 137, 138, 141, 146, 149, 151, 153, 156, 157, 158, 170/190, 171, 172, 174, 176, 177, 178, 179, 180, 183, 187, 194, 195, 196/203, 201, 200, 202, 206, 207, and 208 (International Union of Pure and Applied Chemistry numbers) [28].

The quantitative analysis of organochlorine compounds was performed with capillary gas chromatography, coupled with a mass selective detector operated in selected ion monitoring mode. Each cleaned up sample was injected twice. The first injection was designed to determine the organochlorine compounds by using 21 organochlorine standards. The second injection was to determine PCBs by using Aroclors 1242, 1254, and 1260, at a 1:1:1 quantitation standard mixture. The samples were analyzed with a HP 5890 gas chromatograph No. 1 and HP mass selective detector HP 5971 (Hewlett-Packard, Wilmington, DE, USA). The detection limit was 0.0001 μg/g wet weight. Trace concentrations were between 0.0001 and 0.0009 μg/g wet weight, and were treated as 0 in the statistical analyses. The percent recovery of 13C12-labeled internal standard PCBs ranged from 64.52 to 104.74%, with a mean percent recovery efficiency of 80.24%. The percent recovery of 13C12-labeled internal standard of tetra-, penta-, and hexachlorobenzene ranged from 39.73 to 93.87% with a mean percent recovery of 67.69%. The PCBs and pesticides were not corrected for the percent recoveries.

The variances in organochlorine levels differed among sites, so mean contaminant levels were compared among sites by means of t tests for unequal variances and unequal sample sizes. The eggs from Hamilton had a significantly higher lipid content (t = 3.30, df = 15, p = 0.005) than those from Algonquin. Because a positive relationship existed between lipid levels and the concentrations of the organochlorine compounds (results not shown), the confounding effect of lipid content upon the relative organochlorine concentrations in eggs among sites was reduced by dividing the organochlorine levels by the percent lipid before statistical analyses. Ranking the sites in order of relative contamination by contrasting mean concentrations among sites for each compound is difficult because of the small sample sizes of clutches available within each site, thus resulting in low power. Because of this, a sign test [27] was used to determine if any trend existed in ranking of the means of organochlorine concentrations among sites (i.e., testing if the mean concentrations were consistently higher at some sites).

Mercury concentrations were determined in the same egg subsamples that were analyzed for chlorinated hydrocarbons and several other clutches collected at each site for another study (Hamilton = 14, Algonquin Park = 3, Akwesasne = 8). Eggs were pooled together as a single sample per site, and were freeze-dried and placed in acid-washed test tubes. To each test tube, 0.5 ml of deionized H2O and then 1.0 ml of HNO3 (70%) were added. Volumes were then adjusted to 20 ml. Eggs were capped, at 70°C for approximately 1 h. After cooling, 1.0 ml of H2SO4 (95–97%) and then 0.5 ml of HCl (37%) were added. The tubes were heated again, loosely capped, at 70°C for approximately 1 h. After cooling, the volumes were adjusted to 10 ml with 2 mM K2Cr2O7 in 3% HCl. Volumes were then adjusted to 20 ml with 9.9 ml of 1.5% HCl and 100 μl of octanol. Mercury was analyzed by the cold vapor technique. Cold vapors were estimated by atomic absorption spectrophotometry with a 3030 b-AAS (Perkin-Elmer, Shelton, CT, USA) equipped with a VGA–76 hydride generator (Varian, Walnut Creek, CA, USA) and PSC-55 autosampler (Varian; NAQUADAT 80601 [29]). Muscle and liver of spiny dogfish (Squalus acanthias) were used as reference material for quality control analysis (National
Organochlorines and sexual dimorphism in turtles

Table 1. Mean (standard deviation) carapace length (cm), mass (g), and precloacal length (cm) of hatchling snapping turtles from Hamilton, Akwesasne, and Algonquin Park, Canada

<table>
<thead>
<tr>
<th>Sex</th>
<th>Measurement</th>
<th>Hamilton</th>
<th>Akwesasne</th>
<th>Algonquin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>n</td>
<td>n</td>
</tr>
<tr>
<td>Male</td>
<td>Carapace length</td>
<td>2.90 (0.13)</td>
<td>2.92 (0.13)</td>
<td>2.92 (0.13)</td>
</tr>
<tr>
<td></td>
<td>Mass</td>
<td>8.89 (1.03) A</td>
<td>9.45 (0.63) B</td>
<td>9.37 (1.00) B</td>
</tr>
<tr>
<td></td>
<td>Precloacal length</td>
<td>0.91 (0.09)</td>
<td>0.90 (0.08)</td>
<td>0.91 (0.08)</td>
</tr>
<tr>
<td>Female</td>
<td>Carapace length</td>
<td>2.98 (0.10) A</td>
<td>3.00 (0.138) A</td>
<td>2.91 (0.13) B</td>
</tr>
<tr>
<td></td>
<td>Mass</td>
<td>9.25 (0.56)</td>
<td>9.28 (0.85)</td>
<td>9.07 (1.11)</td>
</tr>
<tr>
<td></td>
<td>Precloacal length</td>
<td>0.84 (0.07)</td>
<td>0.83 (0.08)</td>
<td>0.85 (0.09)</td>
</tr>
</tbody>
</table>

*Mass and precloacal length were not adjusted for carapace length. Similar letters (A, B) indicate no significant differences among sites (p = 0.05).*

Research Council of Canada, Marine Analytical Chemistry Standards Program, Ottawa, ON), and one random egg sample (Akwesasne) was analyzed in duplicate. The theoretical detection limit in the digest under these conditions was 0.2 ppb (0.02 µg/g dry wt in tissue for 0.2 g dry wt of sample). The lowest practical working level was five times the theoretical detection limit (1.0 ppb in the digest or 0.10 µg/g dry wt in tissue). The sensitivity was 0.27 ppb in the digest. The recovery of reference material was within the certified range (93.4 and 102.6% for two samples). The analytical spike recovery was 95 and 100%.

No statistical analyses were performed for the mercury data because the samples were pooled within each site. All contaminants are reported on a wet weight basis, except for mercury, which was reported on a dry weight basis.

**RESULTS**

*Body size and precloacal length*

No differences were found in the mean carapace length among male hatchlings from different sites (F = 1.438, df = 2, 461, p = 0.2384), but male hatchlings from Hamilton weighed significantly less than those from Akwesasne and Algonquin (F = 13.277, df = 2, 456, p < 0.0001; Table 1). Conversely, the mean carapace length of female hatchlings from Algonquin was significantly shorter than females from Hamilton or Akwesasne (F = 8.436, df = 2, 182, p = 0.0003), but no difference was found in body mass among sites (F = 0.995, df = 2, 182, p = 0.3716; Table 1).

With the exception of female hatchlings from Hamilton, the precloacal length increased as carapace length increased for both females and males (Fig. 2). The elevation of the regression line for precloacal length versus carapace length was consistently lower for females than males at all three sites (Table 2), but no differences were found in the slope of the lines between males and females among any of the sites (Table 3), thus indicating that the precloacal length of females was consistently lower than that of males at any given body size.

The slope of the line for females from Hamilton was significantly less than the slopes for females from Akwesasne and Algonquin (Fig. 2A and Table 3). Similarly, the slope of the regression lines of males from Hamilton was significantly less than the slopes of the lines of males from Akwesasne and Algonquin (Fig. 2B and Table 3). These comparisons show that the precloacal length of both males and females from Hamilton was significantly lower than those from Akwesasne and Algonquin (F = 14.47, n = 30, p = 0.001).
Hamilton increased with body size at a slower rate than that of males and females from the other two sites.

The elevation of the regression line for females from Akwesasne was lower than that for females from Algonquin (Table 2), indicating that the precloacal length of Akwesasne females was consistently smaller than that of Algonquin females from for any given body size. Differences in elevation of the regression lines between females from Hamilton and other sites were not meaningful because of the differences in slope (Tables 2 and 3). However, no differences were found among sites in elevation of regression lines for male precloacal length versus body size (Table 2).

**Fluctuating asymmetry**

A positive linear relationship was found between both the left scute length ($r^2 = 0.181$, $F = 61.08$, $df = 1, 277$, $p < 0.0001$) and the right scute length ($r^2 = 0.221$, $F = 78.70$, $df = 1, 277$, $p < 0.0001$) with carapace length. No relationship was found between precloacal length and the difference in the left and right scutes of hatchlings from Algonquin Park (standard deviation [SD] = 0.271 cm, respectively). Thus, the measurement bias was very small (and nonsignificant) compared to the actual measurements. The mean absolute difference of the length between the left and right scutes of hatchlings from Algonquin Park was 0.152 cm (and nonsignificant) compared to the actual measurements. The mean absolute difference of the length between the left and right scutes of hatchlings from Algonquin Park was 0.152 cm (standard deviation [SD] = 0.271 cm, respectively) and from Hamilton was 0.271 cm (SD = 0.076, $n = 176$). No difference was found in the mean rank of the fluctuating asymmetry measurements between male hatchlings from Algonquin and Hamilton (Mann–Whitney U test, $U = 8685.0$, $Z = -0.2167$, $p = 0.8285$, $n = 103$, 173). Similarly, no differences were found in the shape of the distributions of the fluctuating asymmetry measurements between male hatchlings from Algonquin and Hamilton (Kolmogorov–Smirnov two-sample test, maximum negative difference = $-0.1065$, maximum positive difference = 0.0710, $p > 0.05$, $n = 103$, 173).

The variance in scute measurements was significantly different between hatchlings from Hamilton and Algonquin, indicating that hatchlings from Hamilton had a larger range in the degree of symmetry than hatchlings from Algonquin. Both Levene’s test for homogeneity of variances ($F = 5.47$, $p = 0.02$) and Bartlett’s $x^2$ ($233.4$, $p < 0.01$) were significant, and the variance in the difference in the left and right scute measurements was higher in hatchlings from Hamilton compared with those from Algonquin. However, both Levene’s and particularly Bartlett’s tests require normally distributed

<table>
<thead>
<tr>
<th>Hamilton M (0.202)</th>
<th>Hamilton F (0.125)</th>
<th>Akwesasne M (0.308)</th>
<th>Akwesasne F (0.357)</th>
<th>Algonquin M (0.343)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamilton F</td>
<td>$t = 0.6793$</td>
<td>$p = 0.2487$</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>(0.125)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Akwesasne M</td>
<td>$t = 2.4163$</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>(0.308)</td>
<td>$p = 0.0082^*$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Akwesasne F</td>
<td>$t = 2.2222$</td>
<td>$p = 0.0418^*$</td>
<td>$t = 0.5265$</td>
<td>NA</td>
</tr>
<tr>
<td>(0.357)</td>
<td></td>
<td>$p = 0.3004$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Algonquin M</td>
<td>$t = 2.4773$</td>
<td>NA</td>
<td>$t = 0.9541$</td>
<td>NA</td>
</tr>
<tr>
<td>(0.343)</td>
<td>$p = 0.0068^*$</td>
<td>$p = 0.1705$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Algonquin F</td>
<td>$t = 2.4093$</td>
<td>NA</td>
<td>$t = 1.7478$</td>
<td>$t = 0.6471$</td>
</tr>
<tr>
<td>(0.382)</td>
<td>$p = 0.0086^*$</td>
<td>$p = 0.412$</td>
<td>$p = 0.2591$</td>
<td></td>
</tr>
</tbody>
</table>

*NA = not available (differences in elevations were not tested); an asterisk (*) indicates that slopes were significantly different ($p = 0.05$).
observations, and the distribution of the fluctuating asymmetry measurements were significantly nonnormal. Because of the lack of a test that does not require normally distributed observations, these parametric tests were used instead, but because of the lack of normality the p values should be viewed as approximate only.

Chlorinated hydrocarbon analysis

The clutches from Hamilton had pesticides and PCBs levels that were two to at least four orders of magnitude higher than the levels from the clutches from the reference site (Algonquin), and were also typically one order magnitude higher than the clutches from Akwesasne except for PCBs. The clutches from Akwesasne had pesticides and PCB levels that were at least one order of magnitude higher than the levels from the clutches from Algonquin. Statistical analyses were not performed with 10 of the compounds from Algonquin and 3 of the compounds from Akwesasne because those compounds were below the minimum detection level. Eight of 11 organochlorine pesticides were significantly higher at Hamilton than at Snye Marsh, Akwesasne, and all four compounds that were detectable at Algonquin were higher at Hamilton than at Algonquin. In the four cases where compounds were detectable at all three sites, none of the comparisons between Akwesasne and Algonquin were significant (Table 4). The mean rank of the organochlorine concentrations was higher from eggs from Hamilton compared to those from Snye Marsh, Akwesasne (Z = 2.940, n = 14, p = 0.0033). Eggs from Hamilton (Z = 3.474, n = 14, p = 0.0005) and from Snye Marsh, Akwesasne (Z = 3.015, n = 14 [three ties], p = 0.0026) had higher mean ranks of the concentrations of organochlorine compounds than Algonquin Park eggs.

Mercury

Mercury levels obtained for the duplicate analysis of the single egg sample from Akwesasne were both 0.30 μg/g dry weight, whereas the pooled sample from Hamilton contained 0.03 μg/g dry weight, and the pooled sample from Algonquin contained 0.04 μg/g dry weight.

**DISCUSSION**

Both male and female hatchlings had altered sexually dimorphic morphology at Hamilton compared to Algonquin and the moderately contaminated site, Akwesasne. A size-specific alteration in the precloacal area relative to body size seems to occur in hatchlings from Hamilton, with larger hatchlings having relatively smaller precloacal lengths. This suggests that at least some of the difference in the morphology of the adults from contaminated sites compared to uncontaminated sites [19] may be due to differences in embryonic development. Although the mechanism of action was not investigated, at least some of the effects of organochlorine contamination are likely organizational during embryonic development, because the alterations in precloacal length occurring very early in the life cycle are probably permanent. At all sites, the precloacal length of female hatchlings was smaller by a constant amount for any given body size compared to males, demonstrating that this sexually dimorphic trait is likely determined by embryonic organizational effects occurring during or soon after sexual differentiation. Because the relationship between precloacal length and body size (i.e., slope) of male and female hatchlings from Hamilton was reduced compared to hatchlings from the other sites, alterations in embryonic organization seem to be occurring at this heavily contaminated site.

Although the alterations in precloacal length of hatchlings from contaminated sites found in this study may partially explain the differences in precloacal length found in adult snapping turtles [19], this does not preclude changes in the growth rate sometime after hatching, but before sexual maturity. Some of the altered sexually dimorphic traits in adults may occur in an organizational period after hatching. Organizational and activational effects of sex hormones, or their analogs, can be considered as extremes on a continuum [30], and organizational effects may occur even in sexually mature adults [31,32].

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**Table 4.** Mean concentrations (standard deviation) of organochlorine compounds (ng/g wet wt), percent moisture, and percent lipid of snapping turtle eggs from Hamilton, Algonquin Park, and Akwesasne, Ontario, Canada. The sample sizes given (n) are the number of clutches used in the analysis.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Algonquin (n = 3)</th>
<th>Hamilton (n = 14)</th>
<th>Akwesasne (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexachlorobenzene</td>
<td>ND</td>
<td>2.79 (1.53)</td>
<td>1.00 (1.00)</td>
</tr>
<tr>
<td>Octachlorostyrene</td>
<td>ND</td>
<td>1.93 (1.33)</td>
<td>ND</td>
</tr>
<tr>
<td>Heptachlor epoxide</td>
<td>ND</td>
<td>3.50 (1.51) A</td>
<td>0.67 (1.16) B</td>
</tr>
<tr>
<td>Oxychlordane</td>
<td>0.67 (1.16) A</td>
<td>31.71 (10.75) B</td>
<td>4.33 (4.16) A</td>
</tr>
<tr>
<td>cis-Chlorodane</td>
<td>ND</td>
<td>4.29 (1.77)</td>
<td>ND</td>
</tr>
<tr>
<td>cis-Nonachlor</td>
<td>ND</td>
<td>33.36 (9.61) A</td>
<td>2.33 (2.52) B</td>
</tr>
<tr>
<td>trans-Nonachlor</td>
<td>ND</td>
<td>57.57 (28.43) A</td>
<td>4.33 (4.51) B</td>
</tr>
<tr>
<td>p,p'-DDE</td>
<td>1.67 (0.58) A</td>
<td>135.14 (85.67) B</td>
<td>10.00 (6.56) A</td>
</tr>
<tr>
<td>p,p'-DDD</td>
<td>ND</td>
<td>2.29 (1.33)</td>
<td>ND</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>ND</td>
<td>8.21 (2.99) A</td>
<td>3.00 (2.65) B</td>
</tr>
<tr>
<td>Photomirex</td>
<td>ND</td>
<td>19.79 (11.79)</td>
<td>6.00 (7.94)</td>
</tr>
<tr>
<td>Mirex</td>
<td>ND</td>
<td>42.21 (28.06)</td>
<td>13.00 (16.52)</td>
</tr>
<tr>
<td>Total CHC</td>
<td>2.33 (1.53) A</td>
<td>343.79 (156.50) B</td>
<td>44.67 (45.63) A</td>
</tr>
<tr>
<td>Sum PCB</td>
<td>20.33 (10.79) A</td>
<td>2,956.28 (1,448.36) B</td>
<td>3,377.00 (3,265.34) AB</td>
</tr>
<tr>
<td>% Lipid</td>
<td>5.36 (0.58)</td>
<td>6.85 (0.80)</td>
<td>7.25 (2.16)</td>
</tr>
<tr>
<td>% Moisture</td>
<td>80.05 (2.55)</td>
<td>73.80 (4.72)</td>
<td>71.90 (5.76)</td>
</tr>
</tbody>
</table>

* Different letters indicate that the concentrations are significantly different (t test for unequal variances), after dividing concentrations by the percent lipid (p < 0.05). Note that the concentrations listed here are not adjusted by percent lipid levels. ND = nondetectable concentration; DDE = dichlorodiphenyldichloroethane; DDD = dichlorodiphenyldichloroethane; CHC = chlorinated hydrocarbon; PCB = polychlorinated biphenyl.
After hatching, juveniles are exposed to organochlorines through residual concentrations from maternal transfer [33] and through new exposure in their diet. Chronic exposure before and during sexual maturity might affect sexually dimorphic traits through interference with normal endocrine function.

Overall, Hamilton was ranked as a heavily contaminated site, and Snye Marsh at Akwesasne was a moderately contaminated site, whereas Algonquin Park eggs showed only background levels of contamination. Also, dioxin and furan levels in eggs from Hamilton collected in 1990 were much higher than those from Akwesasne [20]. The only other studies that reported PCB levels in snapping turtle eggs approaching or exceeding the levels found in this study were a study conducted at Lynde Creek Marsh in 1989, which had a mean of 1,430 ng/g sum PCBs [20]; a study at Hamilton in 1990, which had 3,575 ng/g sum PCBs [20]; and a study at Turtle Creek in 1999, also within Akwesasne, which had a maximum of 737,683 ng/g sum PCBs in 1999 [34]. Similar trends were found for p,p'-DDE: eggs from Lynde Creek Marsh had 231.8 ng/g of p,p'-DDE in 1989 [20], eggs from Hamilton had 388.8 ng/g of p,p'-DDE in 1990 [20]; and eggs from Turtle Creek had a maximum of 852 ng/g of p,p'-DDE in 1999 [34]. Nevertheless, the levels of organochlorines found in Hamilton in this study are higher than most other regions in the Great Lakes area [20]. Past studies showed that Hamilton and Lynde Creek Marsh had higher levels of dioxins and furans than Akwesasne [20,34], thus these compounds potentially could contribute to the effects on snapping turtles reported here. Although no statistical tests could be performed, mercury levels at Akwesasne were an order of magnitude higher than those at Hamilton or Algonquin. However, the concentrations found in the Akwesasne eggs are considered well below concentrations that are toxic to wildlife [35].

Deformities should indicate a high degree of fluctuating asymmetry, and previous work had indicated that deformities in hatching snapping turtles might be due to organochlorine exposure [20]. Although earlier work found some occurrence of deformities in carapacial scutes, this was not a common deformity [20]. We measured carapacial scutes because they were relatively hard tissues, and thus were a good physical characteristic for repeated measurement. However, the results of this study suggest that fluctuating asymmetry, at least of carapacial features, may not be a very sensitive endpoint at environmental concentrations of contaminants. In summary, previous work indicated that adult snapping turtles from contaminated sites had altered secondary sexual characteristics. Our current work with hatchlings suggested that the alterations previously observed in adults likely are initiated early in development, resulting in permanent organizational changes in morphology. Our results support other studies that found that maternal transfer of organochlorines from exposed females might alter secondary sexual characteristics in offspring [6,7]. Despite the altered sexually dimorphic morphology of hatchlings and adults at Hamilton, no evidence exists that the intrinsic population growth rate is strongly hindered at that site. The population is one of the densest populations ever recorded [36], and females have larger clutch masses relative to body size at Hamilton compared to Algonquin Park [37]. Although snapping turtle populations are highly susceptible to reduced adult survivorship, changes in hatching survivorship have little effect on the intrinsic population growth rate [38].

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REFERENCES


