Impact of Organochlorine Contamination on Levels of Sex Hormones and External Morphology of Common Snapping Turtles (*Chelydra serpentina serpentina*) in Ontario, Canada

Shane R. de Solla,[1] Christine A. Bishop,[2] Glen Van Der Kraak,[1] and Ronald J. Brooks[1]

[1]Department of Zoology, University of Guelph, Guelph, Ontario, Canada; [2]Canadian Wildlife Service, Canada Centre for Inland Waters, Burlington, Ontario, Canada

Recent research has suggested that contaminants in the environment may influence sex differentiation and reproductive endocrine function in wildlife. Concentrations of organochlorine contaminants (total polychlorinated biphenyls, pesticides) were higher in the blood plasma of snapping turtles from contaminated sites than in those from reference sites. The ratio of the precloacal length to the posterior lobe of the plastron (PPR) is sexually dimorphic in snapping turtles. There were significant reductions in the PPR at three contaminated sites versus two reference sites. The magnitude of the response was such that a significantly higher proportion of PPRs of males from a contaminated site (Cootes Paradise) overlapped with those of females than PPRs of males from a reference site (Lake Sasajewun). Observers can incorrectly identify the sex of turtles at the contaminated site based on secondary sexual characteristics alone. Unlike the changes to the morphology, there were few changes in 17β-estradiol or testosterone levels, and where differences occurred, there was more variation among reference sites than between the reference and contaminated sites. Our results suggest that environmental contaminants may affect sexually dimorphic morphology in snapping turtles without affecting circulating testosterone or estrogen levels in the adults. *Key words*: biomonitoring, feminization, morphology, organochlorines, pesticides, snapping turtles, testosterone. *Environ Health Perspect* 106:253–260 (1998). [Online 30 March 1998]
http://ehpnet1.niehs.nih.gov/docs/1998/106p253-260desolla/abstract.html

Aquatic ecosystems have been contaminated with pesticides and industrial chemicals on a global scale (*1*). Organochlorine compounds are of particular significance because of their persistence, tissue specificity, widespread use, and toxicity. Snapping turtles (*Chelydra serpentina*) bioaccumulate organochlorines because of their longevity (*2*) and their trophic status as omnivores. Because organochlorines are typically lipid soluble and are metabolized slowly, they tend to accumulate in lipid-rich tissues of snapping turtles, such as adipose tissue and reproductive organs (*3*). Significant levels of polychlorinated biphenyls (PCBs), organochlorine pesticides, and polychlorinated dibenzodioxins (PCDDs) occur in snapping turtle eggs from contaminated sites in Ontario (*4,5*).

Snapping turtles have temperature-dependent sex determination (TSD), such that males are produced when the eggs are incubated between 22 and 28°C and females are produced at incubation temperatures outside this range (*6*). TSD is mediated through differential hormone action or production (*7*), so hormones have an upstream or causal role in sex determination. Exposure of snapping turtle eggs to 17β-estradiol during incubation at male-producing temperatures produced all females (*8*), whereas exposure to estrogen antiserum caused some masculinization of embryos at female-producing temperatures (*7*). Female differentiation in snapping turtles appears to be primarily estrogen dependent, whereas male differentiation appears to be dependent on the absence of estrogen (*9*).

Many potential endocrine disrupting compounds are present in snapping turtle eggs and may influence sex differentiation (*10*). There is evidence that reptiles are sensitive to endocrine disruptors. Slider eggs (*Trachemys scripta*) exposed to estrogenic PCBs produced females at male-producing temperatures (*11*). Furthermore, the presence of these organochlorines may disrupt normal endocrine function and morphology in sexually mature reptiles. Female alligators (*Alligator mississippiensis*), at a site contaminated from a spill of Dicofol and DDT, had abnormally high concentrations of 17β-estradiol in blood plasma, whereas males had depressed testosterone levels and increased levels of 17β-estradiol. Also, juvenile alligators born after the spill had poorly organized testes and small phalli (*12,13*). Environmental contaminants have also been implicated in demasculinization of Florida panthers (*Felis concolor coryi*) (*14*), in lower sex steroid and reduced male secondary sex characteristics in white suckers (*Catostomus commersoni*) (*15*), and in feminization of California gulls (*Larus californicus*) (*16*).

Feminization in turtles may be expressed by changes in the circulating levels of hormones and changes in sexually dimorphic morphology. The precloacal length is longer in male turtles than in females (*17*) because this is where the penis is located. We examined the penis of a dissected adult male snapping turtle (carapace length = 33.9 cm); the tip of the penis in the relaxed position was within 1.5 cm from the cloacal opening. The precloacal length of male snapping turtles is usually longer than the two posterior plastron lobes, whereas it is shorter in females (*17*). In males, the precloacal area grows in length faster than the carapace, whereas in females, these two areas grow at the same rate (17). If exposure to exogenous endocrine-disrupting compounds during sexual development results in feminization, it may reduce the rate of growth of the precloacal length. Consequently, the ratio of the precloacal length to the posterior lobe (PPR) would be smaller in contaminated turtles.

We tested the hypothesis that snapping turtles from contaminated sites would be feminized when compared to snapping turtles from noncontaminated sites. We predicted that snapping turtles at contaminated sites would have depressed testosterone levels and elevated estrogen levels in their blood plasma and would have smaller PPRs. An alternative hypothesis is that variation in sexually dimorphic morphology and levels of sex steroids is dependent on abiotic factors such as temperature, length of active season, or other climactic factors. We tested this hypothesis by determining if latitude could explain the observed differences in testosterone and estrogen levels and sexually dimorphic morphology.

Materials and Methods

Snapping turtles were caught at five different sites in Southern Ontario. Lake Sasajewun (45°35′N, 78°30′W), which is located at the wildlife research station

Address correspondence to C.A. Bishop, Canadian Wildlife Service, Canada Centre for Inland Waters, 867 Lakeshore Road, PO Box 5050, Burlington, Ontario, Canada, L7R 4A6.

We thank Len Simser (Royal Botanical Gardens), David Lean (National Water Research Institute), the Ministry of Natural Resources, and the Central Lake Ontario Conservation Authority for permission to capture turtles, and we thank Holly Spiro, Melinda Portelli, Nicola Koper, and Meg Krawchuk for field work. Funding was provided by the Great Lakes Action Plan, Environment Canada, the Tri-council Eco-Research Program, and NSERC grant A5990 (RJB).
Received 6 May 1997; accepted 14 January 1998.

(WRS) in Algonquin Provincial Park, is a 45-ha dystrophic lake with no history of industrial or agricultural discharge. Snapping turtle eggs from Lake Sasajewun contain very low to nondetectable organochlorine concentrations (*5*). Jack Lake (44°42′N, 78°02′W) is a dystrophic lake with numerous cottages along the shores but no industry, and it has only background levels of organochlorine contaminants in plankton (*1*). These levels are consistent with Ontario lakes that are contaminated primarily through atmospheric deposition (*18*). Cootes Paradise (43°17′N, 79°53′W), in Hamilton Harbour, is a 45-ha eutrophic wetland adjacent to heavy industry and sewage treatment plants on the shore of Lake Ontario. Lynde Creek Marsh (43°45′N, 78°57′W) in Whitby, Ontario, is a eutrophic marsh and creek that drain into Lake Ontario. Big Creek Marsh (42 31′N, 80 29′W) is a 615-ha shoreline marsh that drains a large, agriculturally based watershed into Lake Erie. Snapping turtle eggs collected in 1989 from Lake Sasajewun had significantly lower concentrations of total PCB congeners and *p*-*p*′-DDE [0.32 and 0.04 µg/g wet weight (ww), respectively] than Cootes Paradise (30.57 and 4.52 µg/g ww) or Lynde Creek Marsh (37.57 and 3.62 µg/g ww), whereas eggs from Big Creek turtles had intermediate concentrations (6.23 and 0.74 µg/g ww) (*4*,*5*). For this study, Lake Sasajewun and Jack Lake were considered control sites, Lynde Creek Marsh and Cootes Paradise were designated as contaminated sites, and Big Creek was considered to be a moderately contaminated site.

Materials and Methods

The morphology of snapping turtles was compared from data collected from Lake Sasajewun in 1987–1991, Jack Lake and Lynde Creek Marsh in 1995, Cootes Paradise in 1994–1995, and Big Creek Marsh in 1986–1987. Snapping turtles were caught with baited or unbaited hoop traps from early May to late August. The traps were left overnight and checked every morning. We trapped 15 female and 5 male snapping turtles at Lake Sasajewun from May 29 to July 22, 5 females and 4 males at Lynde Creek from June 25 to July 7, 14 males and 6 females at Jack Lake from July 25 to Aug 14, and 90 females and 80 males at Cootes Paradise from May 8 to Aug 22. Two observers measured turtles: one at Cootes Paradise, Jack Lake, and Lynde Creek and another at Big Creek and Lake Sasajewun. Because we had separate observers taking measurements at different sites, there was a risk that observer bias could affect our results. However, one of the observers from Lake Sasajewun had measured snapping turtles from Cootes Paradise in 1992, which allowed us to test for this potential bias. We compared measurements from Cootes Paradise between 1992 and 1994–1995. Precloacal length was measured from the posterior of the plastron to the cloaca with the tail stretched into a natural position (*19*). The posterior lobe was measured as the length of the two posterior plastron scutes (Fig. 1). Whenever possible, the males were positively sexed by eliciting eversion of the penis and females were identified by capturing nesting turtles.

In 1995, blood samples were obtained from adult snapping turtles (carapace length >20 cm) (*18*) from Lake Sasajewun, Jack Lake, Cootes Paradise, and Lynde Creek Marsh. Blood samples were taken within an hour from when the turtles were removed from the trap, usually between 0900 and 1100 hr. Approximately 4 ml blood was taken from the caudal vein using 5-ml lithium heparin vacutainers and 22 gauge double-sided needles. The blood samples were stored in a cooler in ice and water from 1 to 5 hr and then centrifuged in a clinical centrifuge for 10 min. The blood plasma was transferred to two cryovials, frozen at -5°C, and later transferred to a -20°C freezer. Plasma testosterone and estrogen levels were measured by radioimmunoassay (RIA) (*20*). The plasma samples were extracted (100 µl) by adding 5 ml diethyl ether to each sample and freezing the sample on dry ice and acetone. The ether phase was removed and evaporated and then reconstituted with assay buffer. Each sample was analyzed in duplicate. Validation of these assays for snapping turtles was based on the demonstration that serial dilutions of turtle plasma were parallel to the standardized curves (not shown).

Chlorinated hydrocarbon analysis. Blood plasma samples were frozen at -20°C until preparation for analyses by capillary gas–liquid chromatography. A total of 9 plasma samples from male snapping turtles from Cootes Paradise, 10 from Jack Lake, 4 from Lake Sasajewun, and 2 from Lynde Creek Marsh, was analyzed for PCBs and pesticides. Blood samples from females were pooled for each site and analyzed as single samples for non-*ortho* PCBs and PCDDs/polychlorinated dibenzofurans (PCDFs). Only samples from females were used because the entire samples from males were used for PCB and pesticide analyses. Six samples from Cootes

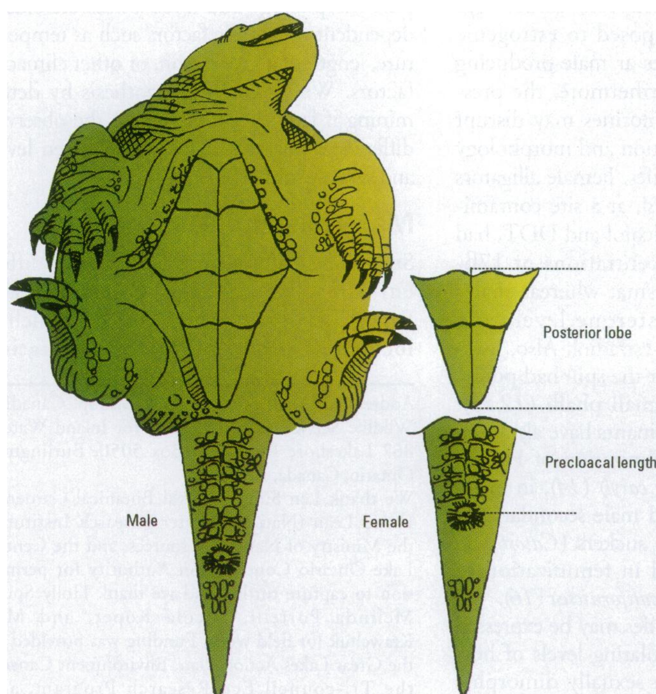

Figure 1. Posterior lobe and precloacal length of male and female snapping turtles.

Table 1. Comparison of CL, carapace length-adjusted PPR, and precloacal length of male snapping turtles among sites

Site	CL (cm)*	PPR**	Precloaca (cm)***
Lake Sasajewan (R) (*n* = 86)	32.67 ± 3.67 AB	1.324 ± 0.173 A	13.80 ± 2.49 AB
Jack Lake (R) (*n* = 14)	32.67 ± 3.43 AB	1.328 ± 0.121 A	14.74 ± 1.98 A
Cootes Paradise (Con) (*n* = 75)	32.90 ± 4.51 B	1.123 ± 0.141 B	13.30 ± 2.76 B
Big Creek (Con) (*n* = 37)	29.86 ± 4.94 A	1.136 ± 0.116 B	12.75 ± 2.46 C
Lynde Creek (Con) (*n* = 3)	31.83 ± 2.48 AB	1.165 ± 0.146 B	13.83 ± 2.26 ABC

Abbreviations: CL, carapace length; PPR, precloacal:posterior lobe ratio; R, reference site; Con, contaminated site; SD, standard deviation. Values of CL are mean ± SD, and PPR and precloacal length are least square mean ± SD. Similar letters (A,B,C) indicate no significant differences among sites (p<0.05).

*ANOVA, $F = 3.84$; $p = 0.0049$.

**ANCOVA, $F = 30.00$; p<0.0001.

***ANCOVA, $F = 8.226$; p<0.0001.

Paradise, four from Lynde Creek, three from Jack Lake, and seven from Lake Sasajewun were pooled for each site. The organochlorine pesticides dichlorodiphenyltrichloroethane (*p,p'*-DDE), 1,2,4,5- and 1,2,3,4-chlorobenzene, hexachlorobenzene (HCB), octachlorostyrene (OCS), photo-mirex (*p*-mirex) and mirex, dieldrin, *cis*- and oxychlordanes, *cis*- and *trans*-nonachlor, heptachlor epoxide, α- and β-hexachlorocyclohexane (HCH), and 37 PCB congeners were measured in the turtle blood plasma using analytical procedures described by Peakall et al. (*21*) with modifications detailed by Turle et al. (*22*) and Bishop et al. (*5*).

The plasma samples were thawed to room temperature and extracted on a column with dichloromethane (DCM):hexane (1:1 v/v). The extract was evaporated to 1–2 ml and extracted on an 8-g 1.2% water-deactivated Florisil column (Floridin Corp., Berkely Spring, WV). This was eluted into three fractions and analyzed separately on a fused silica capillary column (60m × 0.25 mm; 0.1 µm DB-5 film thickness; J&W Scientific, Inc., Folsom, CA). The limit of detection for the organochlorine varied between 0.0003 µg/g ww to less than 0.0001 µg/g ww. The percent lipid content was determined for each sample analyzed; however, due to the small sample size and low lipid content, most of the results for lipid contents were 0%. Only four blood plasma samples from Jack Lake had detectable concentrations of lipids. Although there is a strong relationship between the percent lipid content and lipophilic contaminant concentrations (*23*), we were unable to use lipid content as a covariate. The value of total PCBs reported here is the sum concentration of the following congeners: 44, 49, 52, 60, 66, 74, 87, 99, 101, 105, 110, 118, 128, 129, 137, 138, 141, 146, 149, 151, 153, 158, 170, 171, 172, 174, 180, 182, 183, 185, 194, 195, 200, 201, 203, 206, 1260 [International Union of Pure and Applied Chemistry (IUPAC) number] (*24*). The contaminant analysis of one blood plasma sample was accidently measured twice, so the mean of the two estimates was used for subsequent statistical analyses.

Concentrations of 20 PCDDs, 26 PCDFs, and 6 non-*ortho* PCBs were measured in blood plasma of female snapping turtles collected from all four sites using capillary gas chromatography and mass spectroscopy (*25*). The blood samples were pooled for each site. Initially, samples were extracted by solid-phase extraction using a C18 bonded silica cartridge, but this extraction method was unsuccessful. Thus, liquid/liquid extraction, using Florisil column chromatography, was used instead. Samples were prepared with anhydrous sodium sulfate and extracted with DCM:hexane (1:1 v/v). The sample was spiked with a primary internal standard mixture (^{13}C-12-labeled PCDDs/PCDFs and non-*ortho* PCBs at a level of 50–100 ng/kg on top of the column prior to extraction). Samples were analyzed with a double focusing VG AutoSpec GC/MS (Hewlett-Packard, Wilmington, DE) at 10,000 resolution. The minimum detectable concentration for congeners varied from 0.01 to 0.16 ng/kg ww. Minimum detection limits for non-*ortho* PCBs ranged from 0.01 to 1.05 ng/kg ww. Problems with extraction resulted in sample loss and less than 50% recovery in some cases.

Statistical analyses were performed using Statistica v 5.0 (*26*). The precloacal:posterior lobe ratios (PPR) and precloacal lengths were compared among sites using analysis of covariance (ANCOVA), with carapace length as a covariate to adjust for body size. We used χ^2 analysis to determine if there was a significant overlap of PPR between males and females at Cootes Paradise and at Lake Sasajewun. Regression analysis was used to determine relationships between sex steroid levels and morphology and between sex steroid levels and morphology with latitude. Analysis of variance (ANOVA) was used to determine if there were any differences in mean hormone levels or morphology among sites. Because the blood sampling periods among sites did not completely overlap and body size varied among sites, we used carapace length and Julian date as covariates to eliminate variation in hormone levels due to these confounding factors. The arithmetic means and standard deviations (SD) of PCB and pesticide concentrations were not lipid-normalized because the lipid content was so low as to be unmeasurable for most samples. ANOVA was used to find any differences in PCB and pesticide concentrations among sites. Partial correlations were used to measure relationships between testosterone levels and concentration of organochlorine compounds, both within sites and between sites. Because the estrogen levels were highly variable and sample sizes were so low, the partial correlations between estrogen and organochlorine were spurious; thus, we did not present the results here. We used Spearman rank correlations to test for relationships between the pooled PCDDs/PCDFs and non-*ortho* PCBs from each site and between the mean testosterone and estrogen levels of both male and females for each site. Linear regressions were used to determine if PCB and pesticide concentrations varied with body size. A modified Tukey test for unequal sample sizes was used for *post hoc* multiple comparisons (*27*) for all ANOVAs and ANCOVAs.

This study complied with the University of Guelph animal care protocol.

Table 2. Comparison of CL, carapace length-adjusted PPR, and precloacal length of female snapping turtles among sites

Site	CL (cm)*	PPR**	Precloaca (cm)***
Lake Sasajewun (R) (*n* = 195)	28.13 ± 2.36 AC	0.823 ± 0.09 A	7.71 ± 1.07 A
Jack Lake (R) (*n* = 5)	23.76 ± 6.07 ABC	0.752 ± 0.076 ABC	8.16 ± 0.84 ABC
Cootes Paradise (Con) (*n* = 70)	27.00 ± 2.33 AB	0.647 ± 0.108 B	6.74 ± 1.37 B
Big Creek (Con) (*n* = 156)	25.99 ± 2.74 AB	0.633 ± 0.084 C	6.26 ± 0.86 C
Lynde Creek (Con) (*n* = 5)	28.52 ± 1.07 BC	0.626 ± 0.081 AB	6.33 ± 0.085 ABC

Abbreviations: CL, carapace length; PPR, precloacal:posterior lobe ratio; R, reference site; Con, contaminated site; SD, standard deviation. Values of carapace length are mean ± SD, and PPR and precloacal length are least square mean ± SD. Similar letters (A,B,C) indicate no significant differences among sites (p<0.05).
*ANOVA, F = 18.83; p = 0.0001.
**ANCOVA, F = 80.86; p<0.0001.
***ANCOVA, F = 57.55; p<0.0001.

Results

Morphology. The PPRs of males from the three Great Lakes' sites (contaminated sites) were significantly smaller than those from Jack Lake and Lake Sasajewun (reference sites) (Table 1). There were no significant within-site differences among either the reference sites or contaminated sites. Although there were no significant differences in the PPRs or precloacal lengths between females from Jack Lake and the three contaminated sites, the PPRs and precloacal lengths of females from two of the contaminated sites were significantly smaller than those from Lake Sasajewun (Table 2). Similarly, the precloacal lengths of both sexes were longer at control sites than at most contaminated sites. Only the male PPR was significantly different between Lynde Creek Marsh and the control sites, but since the sample from Lynde Creek Marsh was small, it was difficult to detect a difference.

There were clear differences in the modal distribution of PPR in the turtles between males and females from Cootes Paradise and Lake Sasajewun (Fig. 2). Males had larger

PPRs than females at both sites. A significantly larger proportion of male snapping turtles from Cootes Paradise had PPRs that overlapped female PPRs than did males from Lake Sasajewun (χ^2 = 12.50; p = 0.0004; df = 1) (Fig. 3). The proportion of female PPRs that overlapped male PPRs was not significantly different between Lake Sasajewun and Cootes Paradise (χ^2 = 3.20, p = 0.0737; df= 1) (Fig. 3). When we compared observer measurements within the same site (Cootes Paradise), the observers' bias in measurement error was of a smaller magnitude than the differences in morphology among sites.

Testosterone. Table 3 summarizes the mean testosterone levels at different sites, although two females from Cootes Paradise were excluded as outliers because the estimated testosterone levels were two orders of magnitude larger than the rest. Only at Cootes Paradise were there sufficient observations to examine the relationship between testosterone levels and morphology. Not all observations were used in the following analyses because of occasional missing values from the morphological observations: testosterone levels of males increased linearly with Julian date [adjusted (adj) r^2 = 0.2038; F = 7.401; p = 0.0119; n = 26]; carapace length (adj r^2 = 0.3034; F = 11.888; p = 0.0021; n = 26); precloacal length (adj r^2 = 0.2730; F= 10.390; p = 0.0036; n = 26); and the PPR (adj r^2 = 0.1997; F = 7.478; p = 0.0113; n = 26). However, carapace length, precloacal length, and PPR all had low tolerances (0.04, 0.02, and 0.1, respectively), and thus were intercorrelated. Precloacal length was eliminated to reduce multicollinearity, and the relationship between the remaining factors and testosterone was assessed by a backward stepwise multiple regression. Testosterone had a significant relationship with both carapace length and Julian date (adj r^2 = 0.4957; F= 12.797; p = 0.0002; n = 25).

Julian date (adj r^2 = 0.0518; F = 2.913; p = 0.0970; n = 36), carapace length (adj r^2 = -0.0302; F= 0.0034; p = 0.9503; n = 35), precloacal length (adj r^2 = -0.0228; F = 0.3760; p = 0.5449; n = 29), and PPR (adj r^2 = -0.0361; F = 0.0240; p = 0.8781; n = 29) did not regress significantly with testosterone in females. Again, precloacal length had a low tolerance (0.08) and was eliminated, and a backward stepwise multiple regression showed no relationship between testosterone and carapace length and Julian date (adj r^2 = -0.0355; F= 0.4166; p = 0.6628; n = 35) in females.

Carapace length differed among sites in male snapping turtles (Table 1). Because carapace length and Julian date varied linearly with testosterone levels in males, differences in testosterone due to body size and time could be removed using carapace length and Julian date as covariates. There was a significant difference in mean testosterone levels among sites after adjustment for carapace length and Julian date for males (ANCOVA, F= 5.374; p = 0.0043; n = 32). Testosterone levels of males from Lake Sasajewun were significantly lower than levels of males from Jack Lake, but there were no other differences. Although body size varied among sites for female snapping turtles (Table 2), no covariates were used to compare mean testosterone levels among sites for females because there was no relationship between body size or Julian date with testosterone. There were no significant differences in mean testosterone levels of females (Table 3) among sites (ANOVA, F = 0.3347; p = 0.8003; n = 50).

Estrogen. Table 3 summarizes the mean estrogen levels at different sites. Only at Cootes Paradise were there sufficient observations to compare between estrogen levels and morphology. One male was excluded as an outlier, as his estimated estrogen level was an order of magnitude larger than in any other male. Not all observations were used in the following analyses because of occasional missing values from the morphological observations. In our multiple regression analyses, estrogen levels and all independent variables were transformed, using the reciprocal transformation, to normalize the residuals. There was no relationship between estrogen levels of males and Julian date (adj r^2 = 0.0005; F = 1.0118; p = 0.3245; n = 26), but there was a positive linear relationship between estrogen and carapace length (adj r^2 = 0.1411; F = 5.1076; p = 0.0332; n = 26), precloacal length (adj r^2 = 0.1627; F = 5.8566; p = 0.0235; n = 26), and PPR (adj r^2 = 0.2372; F= 8.7740; p = 0.0068; n = 26). A backward stepwise multiple regression of estrogen with Julian date, carapace length, and PPR eliminated all variables as insignificant except PPR. Precloacal length was removed due to multicollinearity.

There was no relationship between estrogen levels of females and Julian date

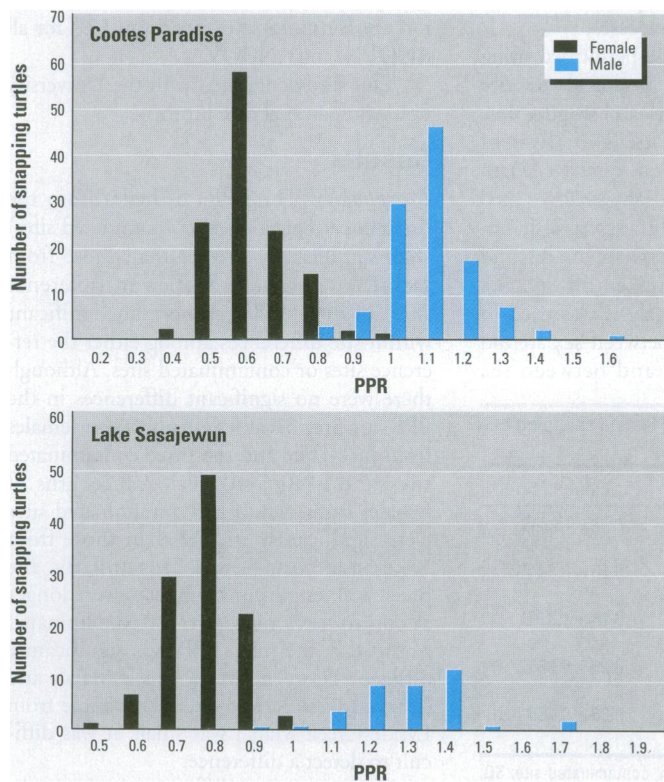

Figure 2. Frequency histogram of precloacal:posterior lobe ratios (PPRs) of snapping turtles at Cootes Paradise in 1994–1995 (males, n = 111; females, n = 125) and at Lake Sasajewun in 1987–1991 (males, n = 39; females, n = 111).

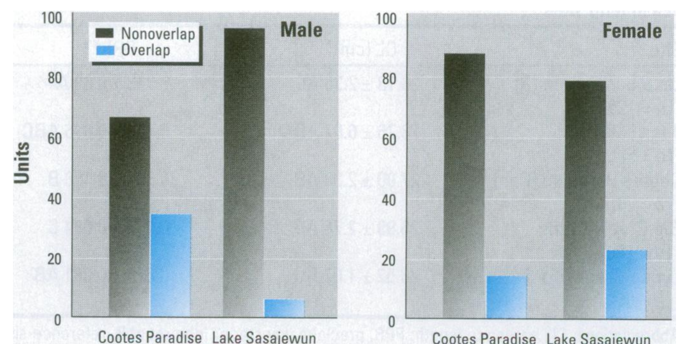

Figure 3. Proportion of male and female precloacal:posterior lobe ratios (PPRs) that overlap with the PPRs of the other sex at Cootes Paradise and Lake Sasajewun.

(adj r^2 = -0.0263; F = 0.3855; p = 0.5408; n = 32), carapace length (adj r^2 = -0.0298; F = 0.1318; p = 0.7192; n = 31), precloacal length (adj r^2 = -0.0393; F = 0.0917; p = 0.7641; n = 25), or PPR (adj r^2 = -0.0404; F = 0.0691; p = 0.7950; n = 25). A backward stepwise multiple regression of estrogen with Julian date, carapace length, and PPR eliminated all variables as insignificant. Precloacal length was removed due to multicollinearity.

Because carapace length and Julian date varied linearly with estrogen for males, carapace length and Julian date were used as covariates to compare mean estrogen levels among sites. There was no difference among sites in estrogen levels of males (ANCOVA, F = 0.8629; p = 0.4331; n = 35). No covariates were used to compare mean estrogen levels among sites for females because estrogen levels were independent of Julian date and carapace length. There was no difference in mean estrogen levels among sites for females (ANOVA, F = 0.4245; p = 0.7364; n = 48).

There was a significant difference in testosterone levels between males and females at Cootes Paradise, where carapace length and Julian date were covariates (ANCOVA, F = 64.0372; p<0.0001; n = 60), but not estrogen (ANCOVA, F = 0.7440; p = 0.3923; n = 57).

Latitude. There was no relationship between levels of testosterone and latitude in either males (adj r^2 = -0.0254; F = 0.0728; p = 0.7888; n = 38) or females (adj r^2 = 0.0024; F = 1.1102; p = 0.2977; n = 47). We transformed the estrogen levels and latitude using the reciprocal transformation prior to regression analysis. There was no relationship between estrogen and latitude for either males (adj r^2 = -0.0182; F = 0.3550; p = 0.5551; n = 37) or females (adj r^2 = -0.0222; F = 0.0248; p = 0.8756; n = 47). In males, there was a significant relationship between latitude and both size-adjusted precloacal length (adj r^2 = 0.0968; F = 23.943; p<0.0001; n = 215) and PPR (adj r^2 = 0.1515; F = 39.213; p<0.0001; n = 215). Similarly, in females, there was a significant relationship between latitude and size-adjusted precloacal length (adj r^2 = 0.0968; F = 23.943; p<0.0001; n = 461) and PPR (adj r^2 = 0.1515; F = 39.213; p<0.0001; n = 461). These regressions were subdivided into contaminated and control sites, but there were no clear trends of either size-adjusted precloacal length or PPR with latitude within these two categories of sites.

Chlorinated hydrocarbons. PCB and pesticide levels were higher in blood plasma of males from Cootes Paradise and Lynde Creek than of males from Jack Lake or Lake Sasajewun (Table 4). There were few differences between either Cootes Paradise and Lynde Creek or between Jack Lake and Lake Sasajewun except for *p,p'*-DDE and total PCB concentrations. However, we had low power to detect any differences due to our small sample sizes. Because there were significant relationships between testosterone concentrations and both carapace length and Julian date, a partial correlation was performed to estimate the effect that PCB and pesticide concentrations have upon testosterone levels at each site. After partialling out the effects of body size and Julian date, there were no significant relationships between testosterone levels and either PCB or pesticide concentrations at either Cootes Paradise or Jack Lake (Table 5). The partial correlations were almost equally divided between positive and negative values. Similarly, there were no significant relationships between mean testosterone levels and either mean PCB or mean pesticide concentrations among all sites, although most of the values were positive.

Although 70.6% of the PCDDs/ PCDFs and non-*ortho* PCBs detected in the pooled samples were higher in blood plasma collected at Jack Lake than at Cootes Paradise and 57.9% were higher at Jack Lake than at Lynde Creek Marsh, the concentrations were of the same magnitude (Table 6). If the concentrations were higher at either Cootes Paradise or Lynde Creek than at Jack Lake, then the concentrations differed by at least an order of magnitude. Generally, the contaminant levels were highest and similar in Cootes Paradise and Lynde Creek Marsh, lower in Jack Lake, and lowest in Lake Sasajewun. Similarly, there were no significant relationships between the pooled PCDDs/PCDFs and non-*ortho* PCBs from each site with the mean testosterone levels of either males or females.

Discussion

Our hypothesis that the sexually dimorphic morphology of adult snapping turtles would be more feminized at contaminated sites was

Table 3. Mean concentrations ± SD of testosterone and estrogen in blood plasma (ng/ml) of snapping turtles from Cootes Paradise, Jack Lake, Lake Sasajewun, and Lynde Creek Marsh in 1995

	Male		Female	
Hormone/site	Mean ± SD	No.	Mean ± SD	No.
Testosterone				
Jack Lake (R)	55.40 ± 16.40	7	0.69 ± 0.01	3
Lake Sasajewun (R)	11.45 ± 6.49	3	0.46 ± 0.12	7
Cootes Paradise (Con)	43.88 ± 21.50	27	0.59 ± 0.32	35
Lynde Creek (Con)	39.38 ± 6.47	2	0.47 ± 0.27	3
Estrogen				
Jack Lake (R)	0.62 ± 0.07	8	0.39 ± 0.11	3
Lake Sasajewun (R)	1.16 ± 0.98	2	0.62 ± 0.38	8
Cootes Paradise (Con)	1.95 ± 2.77	27	2.40 ± 6.25	33
Lynde Creek (Con)	NA	0	0.40 ± 0.11	3

Abbreviations: SD, standard deviation; R, reference site; Con, contaminated site; NA, not analyzed.

Table 4. Mean concentrations ± standard deviation of PCB and organochlorine pesticide (ng/g wet weight) in blood plasma of male snapping turtles from Cootes Paradise, Lynde Creek, Jack Lake, and Lake Sasajewun, Ontario, in 1995

Compound	Cootes Paradise (Con) n = 9[a]	Lynde Creek (Con) n = 2	Jack Lake (R) n = 10	Lake Sasajewun (R) n = 4
1,2,4,5-CB	0.4 ± 0.9 A	ND	0.2 ± 0.4 A	0.7 ± 1.5 A
1,2,3,4-CB	0.2 ± 0.4 A	0.5 ± 0.7 AB	1.0 ± 0.6 B	0.2 ± 0.4 AB
HCB	0.4 ± 0.4 A	0.3 ± 0.1 A	ND	ND
OCS	0.2 ± 0.2 A	0.4 ± 0.1 A	ND	ND
trans-Nonachlor	10.8 ± 11.3 A	6.2 ± 1.6 AB	0.3 ± 0.4 B	0.1 ± 0.1 AB
p,p'-DDE	10.1 ± 4.3 A	21.7 ± 3.0 B	0.7 ± 0.9 C	0.2 ± 0.4 C
p'-Mirex	3.8 ± 3.4 A	5.2 ± 0.9 AB	0.4 ± 0.1 B	0.2 ± 0.3 AB
Mirex	8.3 ± 7.9 A	10.0 ± 2.1 AB	0.4 ± 0.2 B	0.4 ± 0.1 AB
α-HCH	0.5 ± 0.7 A	0.7 ± 1.0 AB	1.4 ± 0.7 B	0.5 ± 1.0 AB
γ-HCH	ND	ND	0.3 ± 0.8	ND
Oxychlordane	7.0 ± 8.8 A	2.4 ± 0.7 A	0.2 ± 0.3 A	0.2 ± 0.3 A
cis-Chlordane	0.8 ± 0.5 A	0.7 ± 0.4 A	ND	ND
cis-Nonachlor	5.3 ± 5.0 A	2.6 ± 0.7 A	ND	ND
Heptachlor epoxide	0.6 ± 1.2 A	0.9 ± 0.2 A	ND	1.5 ± 2.0 A
Dieldrin	0.9 ± 1.2 AB	3.8 ± 1.9 A	0.3 ± 0.8 B	ND
Total PCB	414.8 ± 351.7 A	263.3 ± 116.2 AB	17.8 ± 7.8 B	18.2 ± 12.5 AB

Abbreviations: PCB, polychlorinated biphenyl; Con, contaminated site; R, reference site; CB, chlorinated biphenyl; HCB, hexachlorobenzene; OCS, octachlorostyrene; HCH, hexachlorocyclohexane; ND, not detected.
Similar letters (A,B,C) indicate no significant difference in concentrations among sites (p<0.05).
[a]Number of plasma samples used in analysis.

Table 5. Partial correlations of organochlorine concentrations with testosterone levels in blood plasma of male snapping turtles from Cootes Paradise and Jack Lake after removing the effects of body size and Julian date

Compound	Cootes Paradise (Con)		Jack Lake (R)	
	Partial r^2	*p*-Value	Partial r^2	*p*-Value
1,2,4,5-CB	-0.5224	0.1841	-0.1376	0.8253
1,2,3,4-CB	-0.0671	0.8747	0.3804	0.512
HCB	-0.6995	0.0535	NA	NA
OCS	-0.3254	0.4316	NA	NA
trans-Nonachlor	0.1159	0.7845	0.2862	0.6406
p,p′-DDE	0.401	0.3248	0.112	0.8577
p′-Mirex	0.0759	0.8582	0.78	0.1197
Mirex	0.0763	0.8575	-0.2358	0.7026
α-HCH	-0.5967	0.1184	0.6244	0.2602
γ-HCH	NA	NA	0.616	0.2685
Oxychlordane	0.427	0.2913	-0.0674	0.9142
cis-Chlordane	0.0213	0.96	NA	NA
cis-Nonachlor	0.3438	0.4043	NA	NA
Heptachlor epoxide	0.0754	0.8592	NA	NA
Dieldrin	-0.3868	0.3439	-0.0674	0.9142
Total PCB congeners	0.2434	0.5614	-0.6597	0.2257

Abbreviations: R, reference site; Con, contaminated site; CB, chlorinated biphenyl; HCB, hexachlorobenzene; OCS, octachlorostyrene; HCH, hexachlorocyclohexane; PCB, polychlorinated biphenyl; NA, not applicable.

Table 6. Concentrations (ng/kg wet weight) of non-*ortho* PCB, PCDD, and PCDF congeners in blood plasma of female snapping turtles from Cootes Paradise, Lynde Creek, Jack Lake, and Lake Sasajewun, Ontario, in 1995

Compound	Site			
	Cootes Paradise (Con) $n = 6$	Lynde Creek (Con) $n = 4$	Jack Lake (R) $n = 3$	Lake Sasajewun (R) $n = 7$
Non-*ortho* PCB congener				
PCB-37	2.88	3.87	4.02	2.13
PCB-81	3.61	2.47	0.37	ND
PCB-77	3.16	3.36	4.77	1.78
PCB-126	54.24	57.68	3.27	2.05
PCB-169	2.48	4.57	0.39	ND
PCB-189	ND	0.13	ND	ND
PCDD congener				
2,3,7,8-TCDD	0.73	1.73	0.11	ND
1,2,3,7,8-PentaCDD	0.33	2.64	0.33	0.19
1,2,3,4,7,8-HexaCDD	0.12	0.26	0.42	ND
1,2,3,6,7,8-HexaCDD	0.33	2.38	0.32	ND
1,2,3,7,8,9-HexaCDD	0.1	0.26	0.39	ND
1,2,3,4,6,7,8-HeptaCDD	0.41	0.17	0.67	ND
OctaCDD	0.91	0.86	4.59	0.22
PCDF congener				
1,2,4,7,8-PentaCDF	ND	0.21	ND	ND
1,2,3,7,8-PentaCDF	0.07	ND	0.18	ND
2,3,4,7,8-PentaCDF	0.33	0.88	0.29	ND
1,2,4,6,7,8-HexaCDF	ND	ND	0.07	ND
1,2,4,6,8,9-HexaCDF	ND	ND	0.11	ND
1,2,3,4,7,8-HexaCDF	0.09	0.08	0.23	ND
1,2,3,6,7,8-HexaCDF	0.12	0.34	0.25	ND
1,2,3,7,8,9-HexaCDF	0.14	0.28	0.41	ND
2,3,4,6,7,8-HexaCDF	0.31	0.37	0.56	ND
1,2,3,4,6,7,8-HeptaCDF	0.13	0.15	0.42	ND
1,2,3,4,7,8,9-HeptaCDF	0.09	0.04	0.35	ND
OctaCDF	0.47	0.36	1.31	0.34

Abbreviations: R, reference site; Con, contaminated site; ND, not detected; PCB, polychlorinated biphenyl; PCDD, polychlorinated dibenzodioxin; PCDF, polychlorinated dibenzofuran.
Samples were pooled within each site.

supported. Not only were the precloacal length and PPR smaller at contaminated sites than at reference sites but also the degree of sexual dimorphism was less pronounced at one of the contaminated sites to such an extent that errors in sexing adult turtles using only sexually dimorphic morphology were possible. We have no evidence that any of these turtles whose PPRs overlapped were intersexes, which occasionally occurs after embryonic turtles are exposed to organochlorines (*10*) or hormonal treatment (*7,8*). Many of the males whose PPRs overlapped with females had what appeared to be normal penises. We sexed the turtles with intermediate PPRs by the presence of the penis. The differences in morphology appeared to be better correlated with local levels of contamination than with latitude. If latitude has an effect on morphology, the relationships between PPR or precloacal length would be positive. However, when the regressions between PPR and precloacal length were performed separately within contaminated sites and then within control sites only two of the relationships were positive and six were negative or nonsignificant, suggesting that latitude does not predict morphology.

Our hypothesis that snapping turtles from contaminated sites would have depressed levels of testosterone in blood plasma compared to those from control sites was not supported. In absolute levels, the testosterone concentrations were approximately equivalent at Cootes Paradise and Jack Lake, and the lowest values were at the second reference site (Lake Sasajewun). We were surprised that males from Lake Sasajewun had lower testosterone levels than those from Jack Lake, especially because both are reference sites and Jack Lake was the closest study site to Lake Sasajewun. However, the sample size of testosterone measurements of males from Lake Sasajewun was small, so the mean estimate may not have been reliable. Although it is rare that relationships between hormone levels and contaminant concentrations are found within sites, differential sex steroid levels have been found among sites with different contaminant levels in fish and reptiles (*12,13,28*). However, differences in activity patterns could confound comparisons between northern less-contaminated sites, and southern more heavily contaminated sites. Snapping turtles emerge from hibernation and become active sooner in southern populations (*29*). Testosterone levels have been correlated with mating activity in snapping turtles (*30*) and estrogen levels have been correlated with the ovarian cycle (*31–33*); thus, activity levels, and hormone levels, may be asynchronous between the populations of different latitudes. An implicit assumption of the analysis of covariance is that the regression coefficients (i.e., the relationship of time with hormone levels) are the same for each treatment. Latitudinal differences in behavior and physiology make such comparisons difficult.

Our results are at odds with previous studies examining testosterone profiles of male snapping turtles. Testosterone levels of male snapping turtles from Wisconsin peaked in May and then declined until there was a second, smaller peak in October

(*30,33*). We did not find any apparent peak in any of the hormone profiles; instead, the testosterone levels kept increasing until August, when sampling stopped. Other studies have found that estrogen levels in snapping turtles are lowest in June during ovulation and the postovulation phase, and increase again in the fall during vitellogenesis (*31,32*). However, we found that mean estrogen levels were constant over time, probably because we caught female snapping turtles mostly during the ovulatory and postovulatory period.

There was no evidence that contaminants had any effect upon testosterone or estrogen levels. Although many partial correlations were negative, there were no significant relationships between testosterone levels and any of the PCBs and pesticides within either Cootes Paradise or Jack Lake. Similarly, there was no relationship between testosterone or estrogen and either PCBs or pesticides among sites. Surprisingly, although the relationships were all nonsignificant, most were positive, which is the opposite of what was predicted. As expected, the concentration of most of the PCBs and pesticides measured were lower in turtles from Jack Lake or Lake Sasajewun than in those from Cootes Paradise or Lynde Creek. Nevertheless, 12 of the 16 PCBs and pesticides tested for were found, albeit at low concentrations, in the samples from the control sites. We did not find the same trend with PCDDs/PCDFs and non-*ortho* PCBs. Although the pooled levels of PCDDs/PCDFs and non-*ortho* PCBs were lowest at Lake Sasajewun, there were no differences among Jack Lake, Cootes Paradise, or Lynde Creek, and for many of these contaminants, the levels were highest at Jack Lake. These results are likely confounded by pooling samples; thus, we could not adjust our analyses with covariation with body size.

There were significant regressions between carapace length and PCB or pesticide concentrations in 8 of the 17 compounds examined from the Cootes Paradise turtles, and every regression but one (nonsignificant) was positive. Conversely, none of the 13 regressions between carapace length and PCB or pesticide concentrations from Jack Lake was significant. Bishop et al. (*34*) found no relationship between female body size and organochlorine contaminant concentrations in snapping turtle eggs. However, there was a positive relationship between body size and organochlorine concentration in body tissues (*23,35*). We found that larger turtles had higher concentrations of PCBs and pesticides in blood plasma, at least when the level of contamination was already high. This suggests that the trends in organochlorine contamination in blood plasma follow the same trends as do other tissues. Because turtles from Jack Lake did not show a relationship between body size and contaminant concentration, either we did not have sufficient power to detect the relationship or the contaminants were being metabolized or removed at a rate equal to the rate of uptake. A power analysis (*36*) with a medium effect size of f^2 estimated $1-\beta = 0.1911$ [$F(1,8)$ critical = 5.3177; $\lambda = 1.5$] for the regressions between carapace length and PCB and pesticide concentrations. Therefore the nonnegative regressions were inconclusive (*37*), as we did not have enough power to detect a difference unless the effect size was very large.

Our two main results seem contradictory. There is no evidence that testosterone production has been impaired or estrogen production enhanced in snapping turtles with high levels of organochlorine contaminants. However, there is evidence that there is some feminization occurring at the contaminated sites. *p,p′*-DDE inhibits sexual development in rats without affecting plasma testosterone levels (*38*). The concentration of *p,p′*-DDE that could inhibit androgen receptor activity in the rat was 63.6 ng/g ww (*38*), which is of the same magnitude as the mean concentration of *p,p′*-DDE in the plasma of adult Cootes Paradise turtles (10.1 ng/g ww), total DDT in snapping turtle muscle tissue from the Niagara peninsula in 1989 (164.60 ng/g ww) (*23*), and *p,p′*-DDE in snapping turtle eggs from Cootes Paradise in 1991 (5,910 ng/g ww) (*4*). Previous studies have shown that *p,p′*-DDE is an androgen receptor antagonist and raises the possibility that the changes in sexual characteristics could relate to antiandrogenic activity. These concentrations of *p,p′*-DDE are sufficiently high to inhibit androgen receptor transcription ability *in vitro* in rats (*38*). Similarly, PCBs have been shown to induce changes in sexual development without alterations to testosterone levels (*39*). Our study suggests that external sexual development may be more sensitive to exposure to environmental contaminants than testosterone production or metabolism of adults. Continued exposure during sexual development of juveniles may also contribute to alterations in sexually dimorphic morphology, particularly since the dose of the exposure increases as the turtle gets larger.

Biomonitoring often involves either sampling body tissue (*2,5,23*) or eggs (*4,5,21,22*). Unfortunately, although snapping turtles make good environmental indicators, snapping turtle populations are highly susceptible to increased adult mortality (*40,41*). Repeated or large-scale lethal tissue sampling of snapping turtle adults is undesirable, particularly if the population is already in decline. Although measuring contaminant levels in eggs would have a much smaller effect upon the adult population, contaminant levels in eggs may better represent recent dietary intake just prior to egg production (*35*) rather than long-term accumulation in body tissue. Using blood plasma samples as a measure of bioaccumulation may be a preferable method for biomonitoring, as it is nonlethal and probably causes little harm to adults. Although lipid levels were generally undetectable in the plasma samples and contaminant levels are proportional to lipid levels, random error may be introduced, but the estimate would be unbiased using this method. Because contaminant concentrations present in blood plasma are proportional to body size, blood sampling could be used to measure long-term bioaccumulation of contaminants.

References

1. Taylor WD, Carey JH, Lean DRS, McQueen DJ. Organochlorine concentrations in the plankton of lakes in Southern Ontario and their relationship to plankton biomass. Can J Fish Aquat Sci 48:1960–1966 (1990).
2. Olafsson PG, Bryan AM, Bush B, Stone W. Snapping turtles—a biological screen for PCB's. Chemosphere 12:1525–1532 (1983).
3. Bryan AM, Olafsson PG, Stone WB. Disposition of low and high environmental concentrations of PCBs in snapping turtle tissues. Bull Environ Contam Toxicol 38:1000–1005 (1987).
4. Struger JE, Bishop CA, Obbard ME, Norstrom RJ, Weseloh DV, Simon M, Ng P. Environmental contaminants in eggs of the common snapping turtle (*Chelydra serpentina serpentina*) from the Great Lakes–St. Lawrence River Basin of Ontario, Canada (1981, 1984). J Great Lakes Res 19:681–694 (1993).
5. Bishop CA, Ng P, Norstrom RJ, Brooks RJ, Pettit KE. Temporal and geographical variation of organochlorine residues in eggs of the common snapping turtle (*Chelydra serpentina serpentina*) (1981–1991) and comparisons to trends in herring gull (*Larus argentatus*) eggs in the Great Lakes basin in Ontario. Arch Environ Contam Toxicol 31:512–524 (1996).
6. Yntema CL. Effects of incubation temperatures on sexual differentiation in the turtle, *Chelydra serpentina*. J Morphol 150:453–462 (1976).
7. Crews D, Wibbels T, Gutzke WJHN. Action of sex steroid hormones on temperature-induced sex determination in the snapping turtle (*Chelydra serpentina*). Gen Comp Endocrinol 76:159–166 (1989).
8. Gutzke WHN, Bull JJ. Steroid hormones reverse sex in turtles. Gen Comp Endocrinol 64:368–372 (1986).
9. Rhen T, Lang JW. Temperature-dependent sex determination in the snapping turtle: manipulation of the embryonic sex steroid environment. Gen Comp Endocrinol 96:243–254 (1994).
10. Colborn T, vom Saal FS, Soto AM. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ Health Perspect 101:378–384 (1993).
11. Bergeron JM, Crews D, McLachlan JA. PCBs as environmental estrogens: turtle sex determination as a biomarker of environmental contamination. Environ Health Perspect 102:780–781 (1994).
12. Guillette LJ Jr, Gross TS, Masson GR, Matter JM, Percival HF, Woodward AR. Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. Environ Health Perspect 102:680–688 (1994).
13. Guillette LJ Jr, Pickford DB, Crain DA, Rooney AA, Percival HF. Reduction in penis size and plasma testosterone concentrations in juvenile alligators living in a contaminated environment. Gen Comp Endocrinol 101:32–42 (1996).

14. Facemire CF, Gross TS, Guillette LJ Jr. Reproductive impairment in the Florida panther: nature or nurture? Environ Health Perspect 103(suppl 4):79–86 (1995).
15. Munkittrick KR, Portt CB, Van Der Kraak GJ, Smith IR, Rokosh DA. Impact of bleached kraft mill effluent on population characteristics, liver MFO activity, and serum steroid levels of a Lake Superior white sucker (*Catostomus commersoni*) population. Can J Fish Aqua Sci 48:1371–1380 (1991).
16. Fry DM, Toone CK. DDT-induced feminization of gull embryos. Science 231:919–924 (1981).
17. Gibbons W, Lovich JE. Sexual dimorphism in turtles with emphasis on the slider turtle (*Trachemys scripta*). Herpetol Monogr 4:1–29 (1990).
18. MacDonald CR, Metcalfe CD. Concentration and distribution of PCB congeners in isolated Ontario lakes contaminated by atmospheric deposition. Can J Fish Aquat Sci 48:371–381 (1991).
19. Mosimann JE, Bider JR. Variation, sexual dimorphism, and maturity in a Quebec population of the common snapping turtle, *Chelydra serpentina*. Can J Zool 38:19–38 (1960).
20. McMaster ME, Munkittrick MR, Van Der Kraak GJ. Protocol for measuring circulating levels of gonadal sex steroids in fish. Can Tech Rep Fish Aquat Sci 1836 (1992).
21. Peakall DB, Norstrom RJ, Rahimtula AD, Butler RD. Characterization of mixed function oxidase systems of the nestling herring gull and its implications on bioeffects monitoring. Environ Toxicol Chem 5:379–385 (1986).
22. Turle R, Norstrom RJ, Collins B. Comparison of PCB quantitation methods: re-analysis of archived specimens of herring gull eggs from the Great Lakes. Chemosphere 22:201–213 (1991).
23. Hebert CE, Glooschenko V, Haffner GD, Lazar R. Organic contaminants in snapping turtle (*Chelydra serpentina*) populations from southern Ontario, Canada. Arch Environ Contam Toxicol 24:35–43 (1993).
24. Ballschmiter K, Zell M. Analysis of polychlorinated biphenyls (PCBs) by glass capillary chromatography. Fresenius Z Anal Chem 302:20–31 (1980).
25. Norstrum RJ, Simon M. Determination of specific polychlorinated dibenzo-*p*-dioxins and dibenzofurans in biological matrices by gel-permeation/carbon chromatography and gas chromatography–mass spectometry. In: Environmental Carcinogens, Methods of Analysis and Exposure Measurement, Vol 11. Polychlorinated Dibenzo-*p*-dioxins and Dibenzofurans (Rappe C, Buser HR, Doget B, O'Neill IK, eds). Lyon:International Association for Research on Cancer, 1991;281–297 (1991).
26. StatSoft, Inc. Statistica for Windows. General Conventions and Statistics I. Tulsa, OK:StatSoft, Inc., 1995.
27. Spotvoll E, Stoline MR. An extension of the T-method of multiple comparisons to include the cases with unequal sample sizes. J Am Stat Assoc 69:975–978 (1973).
28. Munkittrick KR, Van Der Kraak, Washington GJ, McMaster ME, Portt CB. Response of hepatic MFO activity and plasma sex steroids to secondary treatment of bleached kraft mill effluent and mill shutdown. Environ Toxicol Chem 11:1427–1439 (1992).
29. Ernst CH, Barbour RW, Lovich JE, eds. Turtles of the United States and Canada. Washington, DC:Smithsonian Institution Press, 1994.
30. Mahmoud IY, Cyrus RV, Bennett TM, Woller MJ, Montag DM. Ultrastructural changes in testes of the snapping turtle, *Chelydra serpentina*, in relation to plasma testosterone, Δ^5-3β-hydroxysteroid dehydrogenase, and cholesterol. Gen Comp Endocrinol 57:454–464 (1985).
31. Lewis J, Mahmoud IY, Klicka J. Seasonal fluctuations in the plasma concentrations of progesterone and oestradiol-17β in the female snapping turtle (*Chelydra serpentina*). J Endocrinol 80:127–131 (1979).
32. Mahmoud IY, Cyrus RV, Woller MJ, Bieber A. Development of the ovarian follicles in relation to changes in plasma parameters and $^5\Delta 3\beta$ HSD in snapping turtle, *Chelydra serpentina*. Comp Biochem Physiol 82A:131–136 (1985).
33. Mahmoud IY, Guillette LJ, McAsey ME, Cady C. Stress-induced changes in serum testosterone, estradiol-17β and progesterone in the turtle, *Chelydra serpentina*. Comp Biochem Physiol 93A:423–427 (1989).
34. Bishop CA, Brown GP, Brooks RJ, Lean DRS, Carey JH. Organochlorine contaminant concentrations in eggs and their relationship to body size, and clutch characteristics of the female common snapping turtle (*Chelydra serpentina serpentina*) in Lake Ontario, Canada. Arch Environ Contam Toxicol 27:82–87 (1994).
35. Bishop CA. The snapping turtle (*Chelydra s. serpentina*) as an indicator of organochlorine contamination in wetlands [Master's thesis]. York University, Toronto, Canada, 1990.
36. Faul F, Erdfelder E. GPOWER: a general power analysis program. Behav Res Methods Instrum Comput 28:1–11 (1992).
37. Hayes JP. The positive approach to negative results in toxicology studies. Ecotoxicol Environ Saf 14:73–77 (1987).
38. Kelce WR, Stone CR, Laws SC, Gray LE, Kemppainen JA, Wilson EM. Persistent DDT metabolite *p,p'*-DDE is a potent androgen receptor antagonist. Nature 375:581–585 (1995).
39. Lundkvist U. Clinical and reproductive effects of Clophen A50 (PCB) administered during gestation on pregnant guinea pigs and their offspring. Toxicology 61:249–257 (1990).
40. Congdon JD, Dunham AE, van Loben Sels RC. Demographics of common snapping turtles (*Chelydra serpentina*): implications for conservation and management of long lived organisms. Am Zool 34:397–408 (1994).
41. Cunnington DC, Brooks RJ. Bet-hedging theory and eigenelasticity: a comparison of the life histories of loggerhead sea turtles (*Caretta caretta*) and snapping turtles (*Chelydra serpentina*). Can J Zool 74:291–296 (1996).

Reviews in Environmental Health, 1998
Toxicological Defense Mechanisms

Volume 106, Supplement 1, February 1998

Environmental Health Perspectives publishes monographs on important environmental health topics and an annual review issue as supplements to the monthly journal. The February supplement (Volume 106, Supplement 1), our annual review issue, included topics on endocrine disruption, transgenic animals, disease models, chemical toxicology, confounders of toxicity, and global environmental issues. Also in the February issue we presented a series of papers that explored how toxicological defense mechanisms can be used to enhance our understanding of dose–response relationships and low-dose extrapolation procedures required for risk assessments.

Back issues of Environmental Health Perspectives Supplements are available for purchase.
See http://ehis.niehs.nih.gov/ or call 919-541-3841 for ordering information.
(Volume discounts are available for bulk orders.)