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Shane R De Solla
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Relative Contributions of Organochlorine Contaminants, Parasitism, and Predation to Reproductive Success of Eastern Spiny Softshell Turtles (*Apalone spiniferus spiniferus*) from Southern Ontario, Canada

SHANE R. DE SOLLA,[1] MICHELLE L. FLETCHER[2] AND CHRISTINE A. BISHOP[3,*]
[1]*Canadian Wildlife Service, Environment Canada, Box 5050, 867 Lakeshore Road, Burlington, ON, Canada L7R 4A6*
[2]*Upper Thames River Conservation Authority, 1424 Clarke Road, London, ON, Canada N5V 5B9*
[3]*Canadian Wildlife Service, Environment Canada, 5421 Robertson Road, Delta, BC, Canada V4K 3N2*

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Abstract. We examined hatching success, predation rates, rates of parasitism, sex ratio, and egg viability in eggs of the eastern spiny softshell (*Apalone spiniferus spiniferus*), a threatened species in Canada. Eggs were monitored from three populations, located at Thames River, Rondeau Provincial Park, and the Long Point National Wildlife Area, in southern Ontario, Canada in 1998. Concurrently, we measured organochlorine pesticides, PCBs, dibenzo-*p*-dioxins and furans from eggs from the same nests. Contaminant concentrations in eggs were similar among sites. There was no correlation between hatching success, parasitism and depredation rates, or the proportion of hatchlings that were males with total PCBs or individual pesticides, but there was a positive correlation between egg viability with concentrations in eggs of total PCBs, and with five pesticides. We found no evidence that the reproductive success of softshell turtles was compromised due to organochlorine contamination. The most important factors determining hatching success of eggs was predation, followed by egg viability and parasitism.

Keywords: softshell turtles; organochlorines; eggs; reproduction; parasitism

Introduction

Reptiles are one of the most endangered classes of vertebrates in the world (Gibbons et al., 2000). There is increasing concern that the loss of reptiles is not only associated with habitat loss and persecution of these animals, but it may also be due to the potentially high exposure to contamination and sensitivity of some species to chlorinated hydrocarbons (Hopkins, 2000). However there have been extremely few studies of the reproductive effects of these chemicals on reptiles (Sparling et al., 2000).

Eastern spiny softshell turtles (*Apalone spiniferus spiniferus*) have a very limited and fragmented distribution in Canada. Although the species was once common in wetlands throughout southern Ontario and Quebec (Campbell and Donaldson, 1991), currently only three disjunct populations in Ontario (Long Point National Wildlife Area, Rondeau Provincial Park, Thames River) and one population in Quebec persist. At present, no single cause of the loss of this species has been identified, but due to the decline in number and occurrence of the populations, this species was listed as threatened in Canada (Campbell and Donaldson, 1991).

*To whom correspondence should be addressed:
Tel.: 604-940-4700; Fax: 604-946-7022;
E-mail: cab.bishop@ec.gc.ca

Some of the populations are located near areas of agricultural activity and occur in shoreline wetlands of the Great Lakes, and thus may be exposed to organochlorine compounds. Because softshell turtles are carnivorous (Plummer and Farrar, 1981) and have a long life span (Breckenridge, 1955), their eggs have the potential to contain high levels of organochlorine compounds as found in snapping turtles (*Chelydra serpentina*; Bishop et al., 1998) and American alligators (*Alligator mississippiensis*; Guillette et al., 1994). The only reported measurement of organochlorine compounds in eastern spiny softshell turtles was in 1974, where an egg collected from Lake Erie contained 5.68 μg/g wet weight (ww) Aroclor 1254 : 1260, and 0.72 μg/g ww *p*,*p*′-DDE (Campbell, 1974). This is among the highest concentrations found in turtle eggs to date. PCBs, organochlorine pesticides, and dioxins have been associated with reduced hatching success and altered sexual development in other turtle species (Bergeron et al., 1994; Bishop et al., 1998; de Solla et al., 1998).

In 1997, the hatching success of softshell turtles appeared to be relatively low at Long Point, Lake Erie compared to other sites. Given the reproductive effects associated with organochlorine exposure and the threatened status of the eastern spiny softshell turtle in Canada, this study was initiated in 1998 to determine if the differences in hatching success could be due to contaminant exposure. Organochlorine contaminants were measured in turtle eggs collected from Long Point and two other extant populations in southern Ontario, in 1997 and 1998. We tested the hypothesis that organochlorine exposure correlates with reproductive success of spiny softshell turtles. We determined the relationship between pesticide and total PCB concentrations with hatching success, egg viability and sex ratio of hatchlings. Finally, we determined the contributions of parasitism and predation to hatching success.

Methods

Study sites

There were three main study areas: Thames River, Long Point National Wildlife Area, and Rondeau Provincial Park. A 5-km section of the Thames River (42°55′N, 81°25′W) was monitored downstream of the city of London. Eggs were monitored from two sites from the Thames River; one island site (Deer Island) and one point bar site (Turtle autobahn). Long Point National Wildlife Area (42°32′N, 80°04′W) is a 50 km long sand spit jutting into Lake Erie. There were five nesting sites at the Long Point National Wildlife Area: Long Pond, Tip, Wigeon, Hard Hat, and Duncan's Pond. Rondeau Provincial Park (42°15′N, 81°53′W) consists of a sand spit in Lake Erie. A 3-km section of Rondeau Provincial Park was monitored for nesting.

Data collection

All field data were collected in 1998. At all sites, some nests were protected by a cage to exclude predators (Graham, 1997). There were 59 protected nests at Long Point, 39 at Rondeau, and 45 at Thames River. Both protected and unprotected (natural nests) were monitored for hatching success. Natural nests that were not detected early, and thus were not caged, were found by following hatchling tracks back to the nest. Many natural nests were found completely depredated, but the data was not included in any analyses. All known nests that were not protected at Rondeau were depredated. Nests were checked daily for hatching starting in August. All emerging hatchlings were weighed, measured, sexed, and then released on site. Once a nest had started to hatch, the remaining eggs in the nest were excavated after 3–4 days had passed to allow natural hatchling emergence. After the nest was excavated, the eggs were examined to determine if they were infertile or parasitized.

Each turtle egg was categorized as hatched, viable, parasitized, or depredated. Hatchlings that successfully emerged from both the egg and nest, and left the nest area were considered as successfully hatched. Eggs that did not hatch and were not depredated or parasitized were considered unviable. Eggs or hatchlings that were found dead with signs of parasitism without any signs of predation were considered parasitized. Eggs or hatchlings that were found dead and were partially or fully consumed by a predator, regardless of the presence or absence of parasites, were considered depredated. Eggs or hatchlings that did not fit any of these categories ($n = 38$) were not included in our analyses. Only one category was given to each egg.

The only parasites that we detected were sarcophagid flies that are currently unidentified beyond family group (Fletcher, 1999). Flies have been observed to burrow into the sand at nests during the egg hatching period. After a few flies were captured and placed into jars, they began to deposit live maggots within a few minutes. We believe that the flies are able to detect the nests soon after the first turtle eggs start to hatch, and then burrow to the nest to deposit live maggots, which enter the eggs as the turtles begin to hatch. See Iverson and Perry (1994) for a similar account.

Eggs that were not rotten but seemed not to have started developing were designated as infertile, and were removed for contaminant analysis. These eggs were placed in hexane rinsed jars and stored at −5 °C for several weeks and then moved to a −20 °C freezer for several months prior to chemical analysis. Therefore, not all nests in the study were sampled for contaminant analysis. All but two egg samples taken for contaminant analysis were from protected nests, and most were taken in 1998. In 1997, two hatchlings that were found dead in nests were analyzed for contaminants. Analyses from the hatchlings were not used when examining relationships between contaminant burdens and egg fate. Only eggs from protected nests were used to determine relationships between egg fate and contaminant levels.

The sex of hatchlings was determined by examining the shell pattern. The sex of adult spiny softshell turtles can be determined using the spotting pattern of the carapace, but hatchlings have similar carapacial patterns as adults (Graham and Cobb, 1998). In 1995, any hatchlings that did not successfully hatch were dissected, and the sex was identified by examining gonadal structure, which was then compared to the shell pattern. This technique indicated that agreement between the sex designated by examination of gonads and carapace was high.

Organochlorine pesticides and PCBs

Organochlorine pesticides and PCBs were measured in 23 clutches, and the contents of one to four eggs were pooled from each clutch for analysis. The quantitative analysis of organochlorine compounds was performed using capillary gas chromatography. For details of the contaminant analysis, see Bishop et al. (1998). The detection limit for PCBs and organochlorine pesticides was 0.1 ng/g ww, and trace levels were between 0.1 and 0.9 ng/g ww, which were treated as 0 in the statistical analyses. Sixteen pesticides were measured (see Table 1), and the total PCBs is the sum of 59 congeners. The mean percent recovery of PCBs from all sites was 86.53% and ranged from 79.8% to 93.6%. The mean percent recovery of tetra-, penta- and hexa-chlorobenzene from all sites was 81.22% and ranged from 70.7% to 89.6%. The reported concentrations of pesticides and PCBs were not corrected for the percent recoveries. All concentrations are reported on a wet weight basis unless otherwise stated.

Polychlorinated dioxins, furans, and non-ortho polychlorinated biphenyls

Concentrations of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzodioxins (PCDFs) and six non-*ortho* PCBs were measured in one pool of turtle eggs per site (Table 2). Eggs from two to four clutches were used in each pool. Levels of PCDD/F and non-*ortho* PCBs were estimated using capillary gas chromatography and mass spectroscopy. For details, see Bishop et al. (1998). Minimum detection limits were approximately 0.2 ng/kg. The percent lipid content for each subsample was determined by homogenizing 1–2 ml sample with 4 ml of hexane, and the mixture was centrifuged to isolate the hexane layer. The hexane was dehydrated with anhydrous Na_2SO_4, evaporated, and then the remaining lipids were weighed. The mean percent recovery of polychlorinated dioxins and furans of samples from all sites was 68.11%, and ranged from 53% to 86%. The mean percent recovery of non-*ortho* PCBs of samples from all sites was 82.5% and ranged from 54% to 169%.

Toxic equivalency concentrations (TEQs) were calculated for PCBs, non-*ortho* PCBs, PCDDs and PCDFs using congener-specific toxic equivalency factors (TEFs). Since TEFs have not been estimated for turtles, we used TEFs estimated from a variety of animals (Ahlborg et al., 1994; Safe, 1994; Zabel et al., 1995; Kennedy et al., 1996).

Statistics

An ANCOVA was used to compare pesticides and PCBs among locations within Long Point, and among sites using percentage of lipid as a covariate, followed by Fisher's PLSD *post hoc* test. Kendall's coefficient of concordance was used to determine if the non-*ortho* PCBs and dioxin/furan profiles were similar among sites. The coefficient of concordance tests if there was an association among the relative rankings of

Table 1. Mean concentrations (s.d.) of organochlorine pesticides (ng/g ww) and polychlorinated biphenyls (μg/g ww), % moisture, and % lipid of spiny softshell eggs from three sites in southern Ontario. One to four eggs from each clutch were used in the analysis. Minimum detection limits were less than 0.1 ng/g ww

	Thames River	Long Point	Rondeau
Number of clutches	$n = 5$	$n = 13$	$n = 5$
Pentachlorobenzene	0.10 (0.22)	0 (0.00)	0 (0.00)
α-hexachlorocyclohexane	0.80 (0.73)	0.25 (0.89)	0.66 (0.96)
Hexachlorobenzene	0.44 (0.38) A	0 (0.00) B	0.22 (0.15) A
γ-hexachlorocyclohexane	0 (0.00)	0 (0.00)	0.4 (0.89)
Octachlorostyrene	3.22 (1.65) A	0.31 (0.54) B	0.52 (0.50) B
Heptachlor epoxide	2.62 (1.31)	2.68 (2.12)	2.18 (1.13)
Oxychlordane	13.04 (7.20)	6.35 (5.85)	5.1 (1.78)
Cis-chlordane	0.26 (0.37)	0 (0.00)	0.14 (0.31)
Trans-nonachlordane	27.88 (12.67)	21.50 (25.21)	15.64 (11.65)
p,p′-DDE	267.80 (128.03)	208.90 (162.74)	168.40 (83.93)
Dieldrin	5.88 (4.08)	3.36 (5.20)	11.26 (7.50)
p,p′-DDD	0.74 (0.33) A	0.92 (0.76) A	3.82 (1.64) B
Cis-nonachlordane	5.50 (2.55)	4.79 (6.10)	4.16 (3.32)
p,p′-DDT	3.56 (1.94)	1.77 (0.79)	2.28 (0.43)
Mirex	1.96 (1.22)	1.74 (2.76)	1.22 (0.61)
Photomirex	0.68 (0.95)	0.08 (0.31)	0 (0.00)
Total PCBs	1.49 (0.94)	0.78 (0.84)	0.77 (0.53)
Aroclor 1254:1260	2.67 (1.60)	1.41 (1.54)	1.35 (0.97)
Aroclor 1260	1.14 (0.69)	0.58 (0.63)	0.55 (0.34)
% Lipids	8.50 (1.32)	6.86 (1.52)	7.22 (3.96)
% Moisture	66.75 (11.22)	72.64 (4.67)	72.70 (6.62)

Cases where no letters are indicated means there were no significant differences among sites. Differences in letters indicate significant differences in concentrations among sites using ANCOVA with % lipid as covariate ($p = 0.05$).

non-*ortho* PCBs and dioxins/furans among sites; i.e., if relative concentration of each congener within each site was similar among sites.

Odds ratios were calculated to compare the relative likelihood of an event occurring (such as depredation) in a site relative to other sites. The odds ratios were transformed using the natural logarithm (log-odds), and 95% confidence limits of the log-odds were estimated by calculating the 2.5th and 97.5th percentiles based upon the normal distribution (Reynolds, 1977). The log-odds ratios were considered significantly different from zero when the 95% confidence limits excluded 1.0 (Sokal and Rohlf, 1995).

Spearman rank correlations were used to determine the correlation between hatching success, egg viability, parasitism and depredation rates, and the proportion hatchlings that were males with total PCBs and with individual pesticides. All sites were pooled because of the small sample size at each site. Pesticides were only included in the analysis if the majority of the observations were non zero.

Results

Comparisons of contaminants among locations within a site

Using only data from the locations within Long Point, there was a positive relationship between percentage lipid and heptachlor epoxide ($r^2 = 0.308$, $F_{1,11} = 4.90$, $p = 0.0489$), *cis*-nonachlordane ($r^2 = 0.519$, $F_{1,11} = 11.86$, $p = 0.0055$), and *trans*-nonachlordane ($r^2 = 0.358$, $F_{1,11} = 6.14$, $p = 0.0307$), so percentage lipid was used as a covariate in further statistical analysis. There was a difference in the mean concentration of *cis*-nonachlordane among locations in Long Point ($F_{3,8} = 6.206$, $p = 0.0175$). Long Pond had a higher mean concentration of *cis*-nonachlordane (mean = 10.08 μg/g ww) compared to Tip (mean = 1.87 μg/g ww), Hard Hat (mean = 4.77 μg/g ww) and Wigeon (mean = 0.07 μg/g ww). There were no differences in total PCBs among locations in Long Point ($F_{3,8} = 1.78$, $p = 0.2289$). No tests were preformed among

Table 2. Concentrations (ng/kg ww) of non-*ortho*-PCB, PCDD and PCDF congeners in pooled spiny softshell egg samples/hatchlings from three sites in southern Ontario in 1998 and 1997. The number of eggs from each clutch used ranged from one to three eggs, and two to five clutches were pooled for each site

	Thames River*	Long Point	Rondeau
No. in pool	2	4	3
Year collected	1997	1998	1998
% Lipid	5.62	7.32	8.27
% Moisture	76.68	75.96	75.72
Non-*ortho* PCBs			
PCB-37	6.92	2.13	4.90
PCB-81	47.74	37.87	48.66
PCB-77	521.17	258.45	640.52
PCB-126	264.78	281.88	347.4
PCB-169	10.03	18.25	22.26
PCB-189	7.34	8.63	9.26
PCDDs			
2378-T4D	0.94	1.08	1.98
12378-P5D	1.80	1.48	2.22
123478-H6D	0.40	0.35	0.45
123678-H6D	0.86	0.88	1.45
123789-H6D	0.12	0.12	0.16
1234678-H7D	0.42	0.33	0.38
12346789-OD	0.76	0.81	0.86
PCDFs			
2378-T4F	4.68	6.04	12.98
2367-T4F	0.05	0.07	0.14
12468-P5F	0.04	0.08	0.07
12478-P5F	0.10	0.21	0.18
12378-P5F	0.56	0.69	0.99
23478-P5F	1.83	1.73	2.82
123468-H6F	ND	0.03	ND
124678-H6F	ND	ND	0.02
124689-H6F	0.05	0.04	0.03
123478-H6F	0.06	0.05	0.06
123678-H6F	0.21	0.16	0.18
123789-H6F	0.11	0.12	0.13
234678-H6F	0.33	0.32	0.40
1234678-H7F	0.13	0.10	0.11

Residue levels were corrected for % recoveries, since they were calculated by the internal standard quantisation method. Congener nomenclature based on International Union of Pure and Applied Chemistry (IUPAC) numbers (Ballschmiter and Zell, 1980).
*Samples used were hatchlings instead of egg contents.

locations within other sites because of the small sample size.

Comparisons of contaminants among sites

There was a positive relationship between percentage lipid and the concentrations of most pesticides, but not PCBs. There were no differences in the mean concentration of total PCBs among sites ($F_{2,19} = 0.55$, $p = 0.5845$; Table 1). There were few differences in pesticide levels among sites (Table 1). The mean concentration of hexachlorobenzene was lower at Long Point compared to Thames River and Rondeau ($F_{2,19} = 7.22$, $p = 0.0047$; Table 1), the mean concentration of octachlorostyrene was higher at Thames River compared to Long Point and Rondeau ($F_{2,19} = 14.49$, $p = 0.0002$; Table 1), and Rondeau had a higher mean concentration of p,p'-DDD than both Thames River and Long Point ($F_{2,19} = 19.81$, $p < 0.0001$; Table 1).

The relative concentrations of individual non-*ortho* PCBs and PCDD/PCDF congeners were similar among sites (non-*ortho* PCBs: $W = 0.9746$, $\chi^2_5 = 14.62$, $n = 3$, $p = 0.0012$; PCDD/PCDFs: $W = 0.9819$, $\chi^2_2 = 58.92$, $N = 21$, $p < 0.0001$). Thus, the profiles of both non-*ortho* PCBs and PCDD/PCDFs in the eggs of turtle eggs were very similar among sites (Table 2).

There were no differences in the mean TEQ's for total PCBs among sites (Table 3), based either upon TEFs calculated by Kennedy et al. (1996) ($F_{2,20} = 2.47$, $p = 0.1104$), or upon TEFs calculated by Ahlborg et al. (1994) ($F_{2,20} = 0.23$, $p = 0.7969$). Although no tests were performed to compare TEQs for non-*ortho* PCBs or PCDD/PCDFs among sites, the total TEQs were similar among sites. The primary source of TEQs for all sites was *non*-ortho PCBs, with PCDDs and PCDFs having similar contributions (Table 4). Total PCBs contributed very little to the TEQs (Table 3).

Fate of eggs in protected nests

Hatching success of protected nests varied among locations in Long Point ($\chi^2_4 = 76.11$, $p < 0.0001$) but not Thames River ($\chi^2_2 = 2.05$, $P = 0.1523$). When all locations within sites were pooled, hatching success varied among sites ($\chi^2_2 = 319.19$, $p < 0.0001$). Hatching success of protected nests was lower at Rondeau than either Long Point or Thames River (Table 5 and 6).

The proportion of viable eggs from protected nests varied among locations at Long Point ($\chi^2_4 = 10.67$, $p = 0.031$) but not Thames River ($\chi^2_2 = 2.06$, $p = 0.1512$). When all locations within sites were pooled, hatching success varied among sites ($\chi^2_2 = 18.32$, $p = 0.0001$). Eggs from Long Point were 2.93

Table 3. 2,3,7,8-TCDD equivalents (ng/g ww) of total PCBs in spiny softshell turtle eggs from southern Ontario

	Thames River	Long Point	Rondeau
Number of clutches	$n = 5$	$n = 13$	$n = 5$
Kennedy et al. (1996)	0.778 (0.493)	0.344 (0.379)	0.338 (0.281)
Ahlborg et al. (1994)	0.038 (0.022)	0.053 (0.133)	0.016 (0.011)

Sources for toxic equivalency factors are given in the table.

Table 4. 2,3,7,8-TCDD equivalents (ng/kg ww) in pooled spiny softshell turtle eggs from non-*ortho* PCBs, PCDDs and PCDFs

	Kennedy et al. (1996)	Ahlborg et al. (1994)	Safe (1994)	Zabel et al. (1995)	WHO
Non-*ortho* PCBs					
Thames	104.82	26.85	32.19	1.43	26.84
Long Point	100.26	28.51	31.69	1.47	28.50
Rondeau	133.61	35.29	42.26	1.87	35.28
PCDDs					
Thames	2.92	n/a	1.93	2.28	1.84
Long Point	2.71	n/a	1.91	2.18	1.82
Rondeau	4.42	n/a	3.24	3.64	3.09
PCDFs					
Thames	5.15	n/a	1.44	0.82	0.47
Long Point	6.64	n/a	1.53	0.83	0.6
Rondeau	14.28	n/a	2.78	1.43	1.3
Total TEQ					
Thames	112.89	26.85	35.56	4.53	29.15
Long Point	109.61	28.51	35.12	4.48	30.92
Rondeau	152.31	35.29	48.28	6.93	39.67

Table 5. Proportion (%) of eggs that hatched, or were viable, parasitized, depredated, or were male, from protected and natural nests from three sites in southern Ontario

	Thames	Long Point	Rondeau
Protected nests			
Hatched	85.42 (96)	70.77 (1105)	30.86 (729)
Viable	98.82 (85)	89.17 (796)	96.20 (237)
Parasitized	4.17 (96)	9.94 (1167)	3.57 (729)
Depredated	7.29 (96)	2.49 (1167)	61.18 (729)
Male	51.22 (82)	50.99 (557)	46.94 (98)
Natural nests			
Hatched	61.04 (110)	47.27 (598)	n/a
Viable	100.00 (53)	84.83 (356)	n/a
Parasitized	19.70 (66)	3.35 (626)	n/a
Depredated	0.00 (66)	33.33 (669)	100

The proportion of eggs that hatched, or were parasitised or depredated do not add to 1.0 because of missing values for eggs whose development was not accounted for (less than 5% of the samples). Sample sizes as the number of eggs used in the statistical analysis are given in parentheses. All natural nests found at Rondeau were depredated.

Table 6. Odds ratios for hatching success, viability, parasitism, depredation, and sex determination of spiny softshell eggs/hatchlings from protected nests from three sites in southern Ontario

	Odds ratio		
Site	Long Point	Rondeau	Thames
Hatched			
Long Point	n/a	0.18*	2.35*
Rondeau	—	n/a	12.73*
Thames	—	—	n/a
Viable			
Long Point	n/a	0.34*	0.15*
Rondeau	—	n/a	0.43*
Thames	—	—	n/a
Parasitized			
Long Point	n/a	0.34*	0.44*
Rondeau	—	n/a	1.29*
Thames	—	—	n/a
Depredated			
Long Point	n/a	60.78*	3.23*
Rondeau	—	n/a	0.05*
Thames	—	—	n/a
Male			
Long Point	n/a	0.85	1.01
Rondeau	—	n/a	1.18
Thames	—	—	n/a

Odds ratios represent the likelihood of an event occurring from the site as compared to another site.
*Significant ($p = 0.05$).

and 6.87 times more likely to be unviable than eggs from Thames River or Rondeau, respectively (Table 6), although viability of the eggs was above 89% for all sites (Table 5).

The proportion of eggs that were parasitized varied among locations at Long Point ($\chi^2_4 = 54.35$, $p < 0.0001$) and Thames River ($\chi^2_2 = 8.35$, $p = 0.0039$). When all locations within sites were pooled, the proportion of parasitized eggs varied among sites ($\chi^2_2 = 28.32$, $p < 0.0001$). Eggs from Long Point were 2.94 times more likely to be parasitized than eggs from Rondeau, and 2.28 times more likely than eggs from the Thames River (Table 6).

The proportion of eggs from protected nests that were depredated varied among locations at Long Point ($\chi^2_4 = 61.07$, $p < 0.0001$), but the relationship at Thames River was not clear ($\chi^2_2 = 3.78$, $p = 0.052$). When all locations within sites were pooled, the proportion of parasitized eggs varied among sites ($\chi^2_2 = 858.49$, $p = 0.0005$). Eggs from Rondeau were 60.78 and 18.79 times more likely to be depredated than eggs from Long Point or Thames River, respectively (Table 6).

The proportion of hatchlings that were male varied among locations at Long Point ($\chi^2_4 = 10.11$, $p = 0.0386$) but not Thames River ($\chi^2_2 = 2.07$, $p = 0.3556$). When all locations within sites were pooled, the proportion of hatchlings that were male did not vary among sites ($\chi^2_2 = 0.63$, $p = 0.7298$; Table 6).

There was no relationship between hatching success, parasitism and depredation rates, or the proportion of hatchlings that were males with total PCBs, but there was a positive relationship between total PCBs and egg viability ($r = 0.49$, $n = 18$, $p = 0.038$). Five correlations between egg fate and pesticides were significant out of 40 comparisons. There was a positive relationship between egg viability and *p,p′*-DDT ($r = 0.49$, $n = 19$, $p = 0.038$), *p,p′*-DDD ($r = 0.69$, $n = 19$, $p = 0.001$), *trans*-nonachlordane ($r = 0.49$, $n = 19$, $p = 0.040$), *cis*-nonachlordane and ($r = 0.56$, $n = 19$, $p = 0.016$), and dieldrin ($r = 0.63$, $n = 19$, $p = 0.005$).

Protected vs. natural nests

Hatching success was lower in natural nests versus protected nests at both Long Point ($\chi^2_2 = 16.71$, $p < 0.0001$), and Thames River ($\chi^2_1 = 32.18$, $p < 0.0001$; Table 5). Egg viability was higher in protected nests versus natural nests at Long Point ($\chi^2_2 = 4.66$, $p = 0.0308$), but there was no difference in egg viability at Thames River ($\chi^2_1 = 0.63$, $P = 0.4281$; Table 5). Rates of parasitism were lower in natural nests compared to protected nests at Long Point ($\chi^2_2 = 25.04$, $P < 0.0001$), but were higher in natural nests compared to protected nests at Thames River ($\chi^2_1 = 10.04$, $P = 0.0015$; Table 5). Predation rates were, as expected, lower for protected nests compared to natural nests at both Long Point ($\chi^2_1 = 341.73$, $P < 0.0001$) and Thames River ($\chi^2_1 = 5.03$, $P = 0.0249$; Table 5).

Discussion

Egg viability was 89% or higher at all sites, and did not coincide with contaminant trends in eggs. Although there were a few differences in pesticide levels among sites (hexachlorobenzene, octachlorostyrene, and *p,p′*-DDD), there were no consistent trends among sites, and there is little indication that any site was substantially more contaminated than any other. Even though PCDD/PCDF concentrations were slightly higher at Rondeau than at either Long Point or Thames River, the differences among sites were not large, and the TEQs were similar among sites.

The largest source of egg mortality for protected nests at all sites was generally predation, which was between approximately 2% and 7% at Thames River and Long Point, but was high at 61% at Rondeau. Predation rates are usually high for nests of other turtle species, but may be highly variable; reported predation rates of turtle nests ranged from 4.5% (*Testudo graeca*; Diaz-Paniagua et al., 1997) to 84.2% (*Kinosternon subrubrum*, *Pseudemys concinna floridana*, and *Trachemys scripta*; Burke et al., 1998), and may occasionally reach nearly 100% (*Chelydra serpentina*; Congdon et al., 1994). Unprotected softshell turtle nests had much higher rates of depredation, and may have approached 100% at Rondeau Provincial Park. There was no relationship between organochlorine concentrations in the eggs and rates of depredation, and thus there was no apparent avoidance of contaminated food by predators.

Parasitism of eggs in protected nests was low among all sites, but was higher at Long Point (9.94%) than Rondeau (3.57%). Rates of parasitism would undoubtedly have been higher except that mammalian predators depredated many of the nests before the eggs could hatch. Parasitism may be facilitated by the emergence of the first hatchlings, whereby the breach in the nest by the first hatchlings would allow sarcophagid flies access to the rest of the freshly hatching turtles (unpublished data). Although rates of parasitism in hosts may increase following organochlorine exposure through the alteration of host resistance (Luebke et al., 1994), we found no relationship between incidence of parasites and total PCBs or pesticide concentrations. Two reasons for the lack of a relationship between contaminants and the incidence of parasites are the relatively low contaminant levels, particularly dioxin, and the method of infestation of hatchlings. The method of parasitism, where maggots burrow into hatchling turtles, likely precludes any immunological response by the hatchling turtles.

Egg viability was lower at Long Point compared to Thames and Rondeau, but egg viability was high at all sites. There was a positive relationship between total PCBs and five pesticides with egg viability. While it seems unlikely that organochlorine exposure would increase egg viability, there was no evidence for any negative effect of PCBs or pesticides upon hatching success. While organochlorine exposure has been associated with reduced hatching success in snapping turtles in Lake Ontario (Bishop et al., 1998), the concentrations found in snapping turtle eggs from contaminated sites in Lake Ontario were higher than found in spiny softshell eggs in this study. Levels of contamination in softshell eggs were more similar to levels in Lake Erie snapping turtle eggs where no reproductive anomalies have been found (Bishop et al., 1998). Furthermore, contamination was similar among sites, which would reduce the statistical likelihood of detecting a reduction in hatching success even if egg viability was depressed.

It appears that the reproductive success of spiny softshell turtles was not compromised due to organochlorine exposure at Long Point, Thames River, or Rondeau Park. The largest source of mortality from oviposition to just after hatching was predation, mostly if not exclusively by mammalian predators, although poaching and destruction of nest by humans were also important factors. Up to 61% of the eggs in protected nests were destroyed by predators. Egg viability was the next largest contributor to hatching success, and up to 10% of the eggs from protected nests or 15% of the eggs from natural nests that were not depredated or parasitized did not hatch. Whether these eggs were not fertilized, died due to fungal, bacterial, or viral infections, or died due to unfavourable temperature or moisture conditions is impossible to determine. There was no evidence that contaminant loads contributed to decreased egg viability. Finally, parasitism had the smallest effect upon hatching success, and up to 10% of the eggs from protected nests were parasitized, although the rates were higher (20%) for natural nests at Thames River. Rates of parasitism, though, did not vary among sites.

Protected nests fared better than natural nests, as expected. Hatching success and egg viability were higher in protected nests than in natural nests, and predation rates were lower in protected nests than in natural nests. This does not include the natural nests that were destroyed that were not monitored; undoubtedly predation rates of natural nests are much higher than reported here.

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