Arch. Environ. Contam. Toxicol. 27, 82–87 (1994)

ARCHIVES OF
Environmental
Contamination
and Toxicology
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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

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Received: 17 September 1993/Revised: 27 December 1993

Abstract. Statistical analyses were used to determine relationships between body size, clutch size and mass, and relative clutch mass and levels of organochlorine pesticides and seven polychlorinated biphenyl congeners in the eggs of adult female common snapping turtles (*Chelydra serpentina serpentina*). No significant correlations were found between body size (body mass, carapace length, carapace width, plastron length) and lipid normalized concentrations of *p,p'*-DDE, mirex, dieldrin, and polychlorinated biphenyl (PCB) congeners (IUPAC): #52, #105, #118, #138, #153, #180, #194, and the sum concentration of those congeners. Small sample size and clumping of data around the mode of the body size values prevented inferences of nonlinear relationships. It was concluded that body size and clutch characteristics are not strong or reliable predictors of the level of contaminants in snapping turtle eggs and that adjustment for those parameters would not reduce variation in contaminant levels among clutches. Other variables such as individual food preferences and/or foraging activities are more likely to cause variation in chemical concentrations among clutches of eggs within a population. In order to reduce interclutch variation in contaminant levels to 38.6–55.9% in snapping turtles, sample sizes of at least 15 clutches per site are recommended.

Concentrations of lipophilic environmental contaminants are strongly influenced by body size and age in mammals (Drescher *et al.* 1977; Gaskin *et al.* 1979; Denton 1980; Honda *et al.* 1983) and fish (Youngs *et al.* 1972; Scott and Armstrong 1972). Such relationships in lake trout (*Salvelinus namaycush*), coho salmon (*Oncorhynchus kisutch*), and walleye (*Stizostedion vitreum*) in the Great Lakes necessitate the collection of a single age or size class of fish for the study of geographic and temporal variation in organochlorine concentrations (Baumann and Whittle 1988; Borgmann and Whittle 1991). In the common snapping turtle (*Chelydra serpentina serpentina*), body mass, clutch size, and clutch mass can vary greatly among females within populations (Congdon *et al.* 1987), and relationships have been found in this species between body mass and polychlorinated biphenyl (PCBs) and organochlorine pesticide concentrations in liver (Hebert *et al.* 1993) and PCBs in fat (Bishop 1990). The strength of those relationships increases with higher hydrophobicity of chemicals (Hebert *et al.* 1993). Relationships between snapping turtle size and concentrations of polychlorinated dibenzofurans and dioxins in their fat have also been suggested (Ryan *et al.* 1986). Statistical comparisons within snapping turtle populations find that organochlorine concentrations among clutches can be highly variable (Bishop *et al.* 1991; Struger *et al.* 1993); however, the effect of body size, age, and clutch characteristics on variation in contaminant levels in their eggs has not been previously studied.

The hypothesis tested was that in a single snapping turtle population the eggs from larger, older female turtles with the largest clutch size and/or mass would contain the highest concentrations of polychlorinated biphenyls and organochlorine pesticides. These results will be important for interpretation of geographic and temporal trends of persistent chemicals in the eggs of this species.

Methods

In 1990, snapping turtle eggs were collected for chemical analysis immediately after oviposition from 15 nests. Nests were located on the shores of Cootes Paradise, a wetland at the extreme western end of Hamilton Harbour in Ontario, Canada [43°45'N, 78°7'W; see also Struger *et al.* (1993) and Bishop *et al.* (1991) for map and description of Cootes Paradise]. Body mass, clutch size and mass, carapace length and width, and plastron length were measured in the 15 female turtles. Due to the lack of significant variation in contaminant levels among eggs in snapping turtle clutches (Bishop 1990), eggs for chemical analysis were collected from each clutch using the following protocol: A five-egg sample was taken that included one of the first five eggs and one of the last five eggs oviposited and

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Table 1. Spearman rank correlation coefficients between body-clutch size measurements and PCB and organochlorine concentrations in eggs from 15 common snapping turtles[a]

	Body mass	Carapace length	Carapace width	Plastron length	Clutch size	Clutch mass	Relative clutch mass
% Lipid	0.08	0.10	0.25	0.11	0.003	−0.158	−0.54
DDE	−0.29	−0.29	−0.23	−0.22	−0.008	−0.26	−0.15
Mirex	0.007	−0.03	0.004	0.10	0.36	0.12	−0.12
Dieldrin	−0.14	−0.14	−0.22	−0.21	−0.11	−0.38	−0.52
PCB #52	−0.19	−0.33	−0.26	−0.32	−0.54	−0.29	−0.41
PCB #105	−0.25	−0.17	−0.05	−0.03	0.15	−0.12	−0.25
PCB #118	−0.15	−0.14	−0.03	−0.03	0.08	−0.03	−0.36
PCB #138	−0.13	−0.16	−0.04	−0.08	0.06	−0.13	−0.25
PCB #153	−0.19	−0.16	−0.09	−0.06	0.16	−0.05	−0.18
PCB #180	−0.17	−0.18	−0.13	−0.17	0.04	−0.1	−0.09
PCB #194	−0.28	−0.14	−0.13	−0.13	0.12	0.0	0.06
Total PCB	−0.19	−0.15	−0.06	−0.08	0.12	−0.09	−0.24
Body mass	1.0						
Carapace length	0.91	1.0					
Carapace width	0.88	0.88	1.0				
Plastron length	0.91	0.85	0.93	1.0			
Clutch size	0.77	0.72	0.79	0.79	1.0		
Clutch mass	0.77	0.81	0.78	0.78	0.93	1.0	

[a]$P < 0.05$

three other eggs arbitrarily selected from the clutch that were not any of the first or last five eggs laid. All eggs were collected by hand. The contents of the eggs were pooled by clutch and stored at −20°C within 12 h of field collection. Dental casts of carapacial scutes of all females were made, and, where possible, the age of each female was determined (Galbraith *et al.* 1986). Relative clutch mass (RCM) was calculated for 12 females as the proportion of clutch mass to body mass. Relative clutch mass is an indication of the relative amount of energy female turtles allocate to produce a clutch of eggs (Iverson and Smith 1993).

Using the method of Norstrom and Won (1985), lipid concentration and the following organochlorine compounds were measured in eggs: *p,p′*-DDE, mirex, dieldrin, and PCB congeners (IUPAC Nomenclature): #52, #105, #118, #138, #153, #180, #194 (Ballschmiter and Zell 1980). Sum concentration of those PCB congeners is referred to as Total PCB in the following text. Those congeners are representative of an increasing range of log octanol:water partition coefficients (K_{ow}) from 5.84 (PCB #52) to 7.80 (PCB #194) (Hawker and Connell 1988). Briefly, analytical methodology involved dehydration of egg homogenates by grinding with a 6:1 excess of anhydrous sodium sulfate and extracted with hexane in a column. The extract was cleaned up and separated into three fractions by Florisil® chromatography. The fractions were analyzed by gas chromatography-electron capture detector, using a 60-m DB-5 capillary column (Supelco Inc.). Fraction 1 contained PCBs, *p,p′*-DDE, hexachlorobenzene (HCB), pentachlorobenzene (QCB), 1234-tetrachlorobenzene (1234-TeCB), 1235/1245-tetrachlorobenzene (1235/1245-TeCB), and mirex. Fraction 2 contained *cis*-chlordane, oxychlordane, *trans*-nonachlor, and beta-hexachlorocyclohexane (b-HCH). Fraction 3 contained dieldrin. Recoveries of those compounds in this method range from 82% to 94% (Norstrom and Won 1985). Detection limits were 0.005 mg/kg for organochlorine pesticides and 0.0025 mg/kg for PCBs.

Body and clutch measurements and lipid weight concentrations (mg/kg wet weight/% lipid) of each chemical were tested for normality, and all were found to be normally distributed except PCB #52. Simple linear and Spearman rank correlation coefficients, and regressions were determined for each combination of variables. Residuals and scatter plots of values and Spearman ranks were visually examined for indications of nonlinear relationships. Multiple regression analyses were then carried out with each chemical as the dependent variable and body or clutch size measurements as the independent variable. Maximum r^2 values were determined for each variable model. All statistical analysis was performed using CMS/SAS and statistical tests were determined as described in Sokal and Rolhf (1981).

Results

Body mass ranged from 2.6 to 8.1 kg, clutch size ranged from 28 to 57 eggs with clutch masses between 285 to 745 g (Appendix 1). Age ranged from 7 to 50+ years among the eight females for which age could be determined. There was a significant and positive correlation among body and clutch measurements (Table 1). Correlation coefficients for four variables (carapace length and width, plastron length and body mass) in linear regression models varied from 0.06 to 0.24. The coefficient of variation in organochlorine concentrations among clutches ranged from 38.6% to 55.9% (Appendix 1). Lipid normalized chemical concentrations were also significantly positively correlated with one another with the following exceptions: PCB #52 was not significantly correlated with any other compound and *p,p′*-DDE was not correlated with dieldrin.

Body and clutch size measurements were not significantly correlated with any chemical levels in eggs (Table 1; Figure 1)

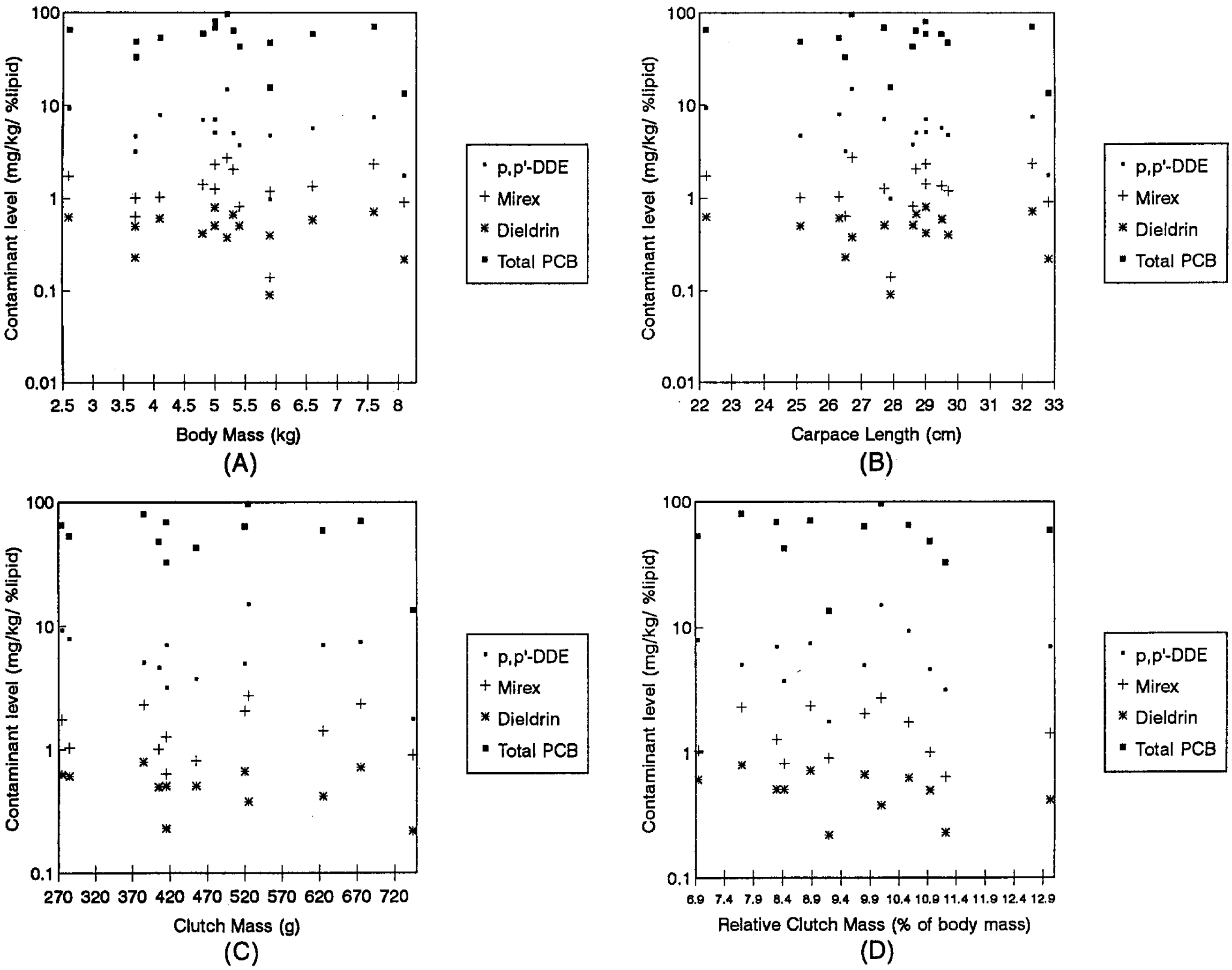

Fig. 1. Body mass (A), carapace length (B), clutch mass (C), and relative clutch mass (D) of 15 female snapping turtles and organochlorine concentrations in their eggs

but correlation coefficients generally indicated weakly negative relationships between body size and chemical (Table 1; Figure 1). Carapace lengths ranged from 22.2 to 32.7 cm, but 80% of the sample lie between 25 and 30 cm. The slightly negative correlations among many body and clutch parameters and contaminant concentrations (Table 1) result from relatively high contaminant levels in eggs of a single, small female (body mass = 2.6 kg).

The distribution of residuals, data, and ranks did not suggest an obvious nonlinear relationship. Multiple regression analysis did not reveal significant relationships between any chemical concentrations in eggs and body and clutch size measures, and maximum r^2 values were consistently low and non-significant (Table 2). The independent variables in the multiple regression analyses were highly correlated with one another, and thus addition of several different measures of body size to the model did little to improve the fit.

Age estimates were possible for only eight females, and, due to the large expected error in estimates, those data were not included in the analysis. However, for this sample of females,

Table 2. r^2 and p values for multiple regression models of body size and clutch size on contaminant levels in common snapping turtle eggs

	r^2 (mass, CL,CW,PL)[a]	p	r^2 (clutch size, RCM)[b]	p
DDE	0.238	0.56	0.001	0.99
Mirex	0.106	0.87	0.234	0.35
Dieldrin	0.065	0.95	0.258	0.93
PCB #52[c]				
PCB #105	0.075	0.93	0.091	0.68
PCB #118	0.069	0.94	0.062	0.77
PCB #138	0.114	0.86	0.017	0.93
PCB #153	0.01	0.89	0.009	0.96
PCB #180	0.115	0.86	0.002	0.99
PCB #194	0.104	0.88	0.005	0.98
Total PCB	0.098	0.89	0.015	0.94

[a]Mass = body mass; CL = carapace length; CW = carapace width; PL = plastron length
[b]RCM = relative clutch mass
[c]PCB #52 is not normally distributed

estimated age appears to generally coincide with the trends in body size and suggests that a significant correlation between age and organochlorine levels would not be expected.

Discussion

The hypothesis that larger, older turtles or those with highest clutch size, mass, or relative clutch mass would produce eggs that are most contaminated with organochlorine contaminants is not supported. Alternately, ecological or physiological parameters such as individual variation in feeding locations, and/or food preferences and metabolism may be possible determinants of variability in contaminant levels among clutches.

In an omnivorous species such as the snapping turtle, feeding location and preferences may more greatly influence contaminent accumulation, and, in the case of Cootes Paradise, food preference would be a more likely factor. Cootes Paradise has relatively low sediment contamination (Semkin and McLarty 1976) whereas certain areas of Hamilton Harbour, to which Cootes Paradise is directly connected, contain sediments and biota highly contaminated with trace metals, PCBs, and polyaromatic hydrocarbons (Harlow and Hodson 1988). Exposure to contaminants might be highly variable among female turtles if differences in foraging locations occurred. For example, among colonial-nesting great blue herons (*Ardea herodia*), differences in foraging locations for individual females, rather than food preferences or wintering areas, are thought to determine variation in organochlorine contaminant levels found among clutches from the same colony (Elliott *et al.* 1992; Harfenist *et al.* 1993). However, for snapping turtles in Cootes Paradise during 1990–1992, radiotelemetry studies of the 15 female snapping turtles examined in this study found that the maximum distance travelled by any female from the nesting site was 2,020 m and home ranges were 0.1–28.4 ha in size. None of the female turtles left Cootes Paradise on any occasion during the 2-year study (Bishop, unpublished data). Therefore, all female turtles in the study were exposed to similarly low levels of sediment contamination within their foraging area. If distance moved represents greater foraging and prey capture rates, certain females may consume more food and thus accumulate higher contaminant levels in their tissues; however, we cannot confirm that increased movement necessarily correlates with higher food consumption.

Snapping turtles are omnivorous, but studies of different populations show that their diet is approximately one-third fish, one-third plant material, and one-third miscellaneous items including amphibians, crustacea, mollusca, birds, bird eggs, sediment, and refuse (Alexander 1943; Coulter 1957). Many of those food items are known to bioaccumulate organochlorine chemicals (Greichus *et al.* 1973; MacDonald and Metcalfe 1989; Metcalfe and Charlton 1990). If distinct differences in food preferences among individual snapping turtles occurs within a population, those snapping turtles that fed more heavily upon fish in Cootes Paradise could be exposed to higher persistent contaminant levels in their food than female turtles that, for example, chose plants and invertebrates, which would generally contain relatively lower organochlorine concentrations relative to fish. At present, there are no diet studies of this nature to confirm or refute this possibility.

Significant relationships occur between body size and concentrations of lipophilic compounds in fat or liver in adult snapping turtles; the lack of such relationships for eggs suggests that levels of chlorinated hydrocarbons in the body tissues are accumulated progressively with size and age, whereas contaminant levels in eggs are not. Those differences coincide with patterns of accumulation and relationships between body mass and contaminant levels in tissues and eggs in birds and fish. In female herring gulls, endogenous fat reserves are not an important source of energy for egg production; rather, diet just prior to egg-laying is the main source (Roudybush *et al.* 1979; Norstrom *et al.* 1986a). There is no evidence that any winter fat is still stored when breeding begins, and fat accumulation prior to yolk formation is probably not necessary since egg weight relative to body weight is only 25%; thus energy requirement for egg production is a modest demand in the annual energy budget for herring gulls (Norstrom *et al.* 1986a). Eggs account for only 12% of the annual excretion of *p,p'*-DDE in herring gulls (Norstrom *et al.* 1986b). In Adelie penguin (*Pygoscelis adeliae*), the transfer rate of female total body burden to clutch is only 4% for both PCBs and *p,p'*-DDE (Tanabe *et al.* 1986). In rainbow trout (*Salmo gairdneri*), where clutch mass relative to body mass is 13.6% (Nimi 1983), mass of visceral and periorbital fat does not decline during egg production when abundant food is available and only 10–15% of the total body burden of 2,2',5,5'-tetrachlorobiphenyl (PCB #52) is excreted via eggs (Guiney *et al.* 1979). As much as 40% of PCB #52 remains in rainbow trout after spawning (Guiney *et al.* 1979).

In snapping turtles from Cootes Paradise, clutch mass accounted for only 6.95–13.0% of body mass of females. The energy required for egg production in snapping turtles is diluted because ovarian follicle enlargement occurs over a 5-month period, and production of shelled eggs requires another several weeks (White and Murphy 1973). With such a small percentage of turtle body mass and energy required to produce eggs, and the annual energy requirements of ectotherms being generally much lower than for birds (Spellerberg 1982), it is probable that lipids for snapping turtle eggs, and consequently lipophilic contaminants in eggs, are derived from daily dietary intake just prior to egg production as in some birds and fish, rather than utilization of stored fats. Thus, it is not surprising that concentrations of organochlorines in snapping turtle eggs are not strongly correlated with size and clutch characteristics of the female snapping turtle, while concentrations in body tissues increase as the turtle ages.

The results of this study show that adjustments for body-clutch characteristics of female snapping turtles during egg collection or statistical analysis of the contaminant data will not reduce variability in organochlorine levels in eggs. Coefficients of variation of chlorinated hydrocarbon concentrations in snapping turtle eggs have been reported as high as 137% when sample sizes ranged from five to ten clutches (Struger *et al.* 1993). Among 15 clutches in the present study, we found coefficients of variation ranging from 38.6% to 55.9%. That level of variation is consistent with those found for other species used as chemical biomonitors in the Great Lakes such as herring gull eggs [calculated from Weseloh *et al.* (1979); N = 10–13/sample site], Atlantic puffin (*Fratercula arctica*), Leach's Petrel (*Oceanodroma leucorhoa*) [Gilbertson *et al.* (1987); N = 5/sample site for each species], and spottail shiners (*Notropis hudsonius*) [calculated from Suns *et al.* (1991); N = 6–10/sample site]. In contrast, coefficients of variation of less than 15% are reported for fish used for biomonitoring such as lake trout and rainbow smelt (*Osmerus mordax*) (N = 12–

Appendix 1. Summary statistics of body mass (kg) and size (cm), clutch size and mass (g), relative clutch mass and organochlorine concentrations (mg/kg lipid weight) in eggs[a]

	Minimum	Maximum	Mean	Standard deviation	Coefficient of variation
Body mass	2.6	8.1	5.3	1.5	27.7
Carapace length	22.2	32.7	28.1	2.6	9.4
Carapace width	20.3	27.2	24.2	1.8	7.5
Plastron length	18.4	25.2	21.7	1.9	8.7
Clutch size	28.0	57.0	43.1	8.0	18.6
Clutch mass	275.0	745.0	477.0	146.6	30.7
Relative clutch mass	6.95	13.0	9.6	1.7	17.7
% Lipid	4.2	9.4	6.0	1.3	21.4
DDE	1.0	14.9	5.9	3.3	55.9
Mirex	1.4	2.7	1.4	0.71	50.7
Dieldrin	0.09	0.8	0.49	0.2	40.8
PCB #52	0.01	0.04	0.02	0.01	50.0
PCB #105	0.47	2.9	1.7	0.7	41.1
PCB #118	2.1	11.7	7.3	2.8	38.6
PCB #138	2.0	16.2	9.3	3.7	39.7
PCB #153	2.5	16.2	9.3	3.7	39.7
PCB #180	1.3	1.3	6.4	3.0	46.8
PCB #194	0.17	1.2	0.61	0.29	47.5
Total PCB	13.3	96.4	54.3	22.3	41.1

[a]N = 15 in all samples except relative clutch mass output for which N = 12

98/sample site) (calculated from Whittle and Fitzsimons 1983), in which adjustment for size and age is made. Therefore, it appears that by collecting samples from 15 clutches of turtle eggs per site, we can expect that common snapping turtle eggs can be used as integrators of contamination on an annual basis in wetland ecosystems with variability comparable to that occuring in colonial waterbird eggs and young-of-the-year minnows but higher than that in smelt and trout from the Great Lakes.

Acknowledgments. We thank the Royal Botanical Gardens and Mr. Len Simser for their cooperation, and Barbara Trumper for field assistance. We also appreciate the careful review and comments of D. V. Weseloh and John Elliott on an earlier version of the manuscript.

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