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Organochlorine Pesticides, PCBs, Trace Elements and Metals in Western Pond Turtle Eggs from Oregon

Abstract

With increased concern over the status of reptile populations globally, contaminant studies should be part of species evaluations. We analyzed eggs of western pond turtles from Fern Ridge Reservoir in western Oregon for 20 organochlorine (OC) pesticides or metabolites, 42 congener-specific polychlorinated biphenyls (PCBs), and 16 trace elements or metals. These eggs represent the first of this species analyzed. The OC pesticides and PCB residue concentrations in the western pond turtle eggs were generally low and similar to those found in eggs of snapping turtles from a remote site in Ontario, Canada. Western pond turtle eggs also contained mercury and chromium, which are metals of special concern. Although few reptilian eggs have been analyzed for metals, the 44.9 μg/g dry weight chromium in a western pond turtle egg in this study may be the highest reported in a reptilian egg. We found no significant difference in contaminant concentrations in eggs from nests in Oregon, where all turtle eggs failed to hatch compared to those where some eggs hatched. During this initial project, however, we were unable to assess fully the role of OCs, PCBs and other contaminants in the western pond turtle decline. Factors other than contaminants may be involved. In another study, snapping turtle eggs near the Great Lakes-St. Lawrence River basin were much more contaminated with evidence reported of effects on sex differentiation and reproductive endocrine function. Egg hatchability, the only reproductive parameter monitored, may not be the most sensitive endpoint. Other endpoints, including endocrine function, deformity rates, growth rates, and sex determination need study.

Introduction

The western pond turtle (*Clemmys marmorata*) is a wide-ranging species that formerly occurred from extreme western Washington, and possibly British Columbia, to northern Baja California, mostly west of the Cascade-Sierra crest (Ernst et al. 1994). Pond turtles are now uncommon in the Willamette Valley north of Eugene, Lane Co., Oregon, but abundance in Oregon increases south of that city where temperatures are higher (Storm and Leonard 1995). Land use in the Willamette basin is 70% forested (primarily in tributary subbasins), 22% agriculture (primarily cropland on the valley floor), and 5% urbanized (Bonn et al. 1995). The basin includes 11 of the 12 largest cities in Oregon, including the 5 largest (Center for Population Research and Census 1992), and approximately 2 million people or 70% of Oregon's population (Oregon Department of Environmental Quality 1988, Bonn et al. 1995). Like other species throughout the world, the western pond turtle has experienced population declines where human numbers or disturbances have increased (Jennings and Hayes 1994, Holland 1994). The U.S. Fish and Wildlife Service was petitioned to list the species under the Endangered Species Act (U.S. Fish and Wildlife Service 1992).

The risk from contaminants in turtle habitat is potentially serious since some western pond turtle populations occur in close proximity to humans (Germano and Bury 2001). Also, since turtles spend much of their lives in or near aquatic habitats, they may be particularly vulnerable to the many contaminants that enter waterways associated with industrial and agricultural land uses. Most data on contaminants in turtles are from snapping turtles (*Chelydra serpentina*) in eastern North America (Meyers-Schöne and Walton 1994, Sparling et al. 2000) in the 1980s and 1990s. These studies revealed highly variable residue concentrations but reported regularly: p,p'-DDE (DDE), p,p'-DDT (DDT), p,p'-DDD (DDD), dieldrin, hexachlorobenzene, heptachlor epoxide, lindane, mirex, photomirex, *cis*-nonachlor, *trans*-nonachlor,

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oxychlordane, and PCBs. Polychlorinated dioxins and furans (PCDDs/PCDFs) were evaluated infrequently. Limited egg residue data were available for painted turtles (*Chrysemys picta*), box turtles (*Terrapene carolina*), and red-eared sliders (*Trachemys scripta*) (Sparling et al. 2000).

Bishop et al. (1991) reported that rates of abnormal development occurred in snapping turtle populations with the highest PCB concentrations in eggs, but rates did not correlate with OC pesticides. However, their study did not statistically consider PCDDs and PCDFs. A reanalysis of the data showed that the occurrences of abnormalities and unhatched eggs were also correlated with several PCDDs and PCDFs (Bishop et al. 1998). In snapping turtles, contaminants may also influence sex differentiation and reproductive endocrine function. The ratio of precloacal length to the posterior lobe of the plastron (PRR) is sexually dimorphic in snapping turtles, and de Solla et al. (1998) found statistically significant reductions in the PRR at three contaminated sites versus two reference sites. A significantly higher proportion of PRRs of males from a contaminated site overlapped with those of females than PRRs of males from a reference site (Lake Sasajewun). Despite changes in morphology, de Solla et al. (1998) reported few changes in 17β-estradiol or testosterone levels in blood plasma.

Since no western pond turtle eggs had been analyzed for contaminants, and contaminant-related problems were reported for other turtle species, the objectives of our study were to: (1) analyze one egg each from a series of 14 clutches from Fern Ridge Reservoir for OC pesticides and metabolites, congener-specific PCBs, and selected trace elements and metals, and (2) evaluate hatching success of eggs from each clutch in relation to residue concentrations in the sample egg collected from that clutch. Hatching success compared to egg residue concentrations on a nest basis provides a means of evaluating possible effects of contaminants on hatching (Blus 1984). When we decided to conduct contaminant studies on a series of western pond turtle eggs, only unhatched eggs were available. Thus, an evaluation of the more sensitive endpoints studied by Bishop et al. (1991) and de Solla et al. (1998) were no longer an option.

Study Area

Fern Ridge Reservoir (ca. 7 km west of Eugene) in the southern Willamette Valley is a wide, shallow lake formed by an earth-fill dam on the Long Tom River, 37.9 km above its confluence with the Willamette River, Lane County, Oregon (Figure 1). The reservoir is drawn down in the fall and winter to provide flood-control and irrigation storage, and refilled by mid-April of each year. Approximately 1,000 ha of land around the reservoir are managed by the US Army Corps of Engineers (COE) for recreation and wildlife habitat. The major tributaries of Fern Ridge Reservoir are Coyote Creek and the Long Tom River, which together drain over 750 km^2 of the eastern Coast Range. The reservoir also receives flows from the Amazon Canal and several minor streams. The Amazon Canal enters the reservoir from the east, and was constructed in 1951 to divert water from Amazon Creek, which drains storm water from Eugene. When full, the reservoir has a surface area of 4,087 ha and is 7.6 m at its deepest point. Average depth is approximately 2.1 m. Emergent plants are abundant in the southern and eastern edges of the lake, along the shoreline and in coves lake-wide. Hundreds of hectares of bulrush (*Scirpus acutus*), reed canary grass (*Phalaris arundinacea*), and cattail (*Typha latifolia*) marsh are found near the mouths of the Long Tom River, Coyote Creek, and Amazon Creek.

We have studied nesting western pond turtles at Fern Ridge Reservoir since 1992. Turtles nest on the western edge of Fern Ridge Reservoir at South Applegate (Site A) and Tripass (Site B) (Figure 1). South Applegate nesting area is a 4 ha native wetland prairie in the southwest corner of the reservoir. The prairie area was plowed and grazed prior to and somewhat after the COE took ownership in 1940, and is now considered a degraded prairie community. However, native wet prairie plants, including tufted hairgrass (*Deschampsia caespitosa*), camas (*Camassia quamash*), and rose (*Rosa nutkana*), are present. Soils are Dayton silt loam with a clay substratum, and drain poorly, which is typical of Willamette Valley wet prairie. Many turtle nest chambers in South Applegate are inundated during the winter by soils saturated with rainwater. Tripass is a 2 ha meadow dominated by short grasses and forbs near a large cove in the northwestern corner of the lake. The area was formerly farmed (pasture and hay), but is currently leased to a local school district for environmental education. The field where nesting occurs is mowed annually in late May prior to turtle nesting. The predominant soil type is Linslaw loam. Although

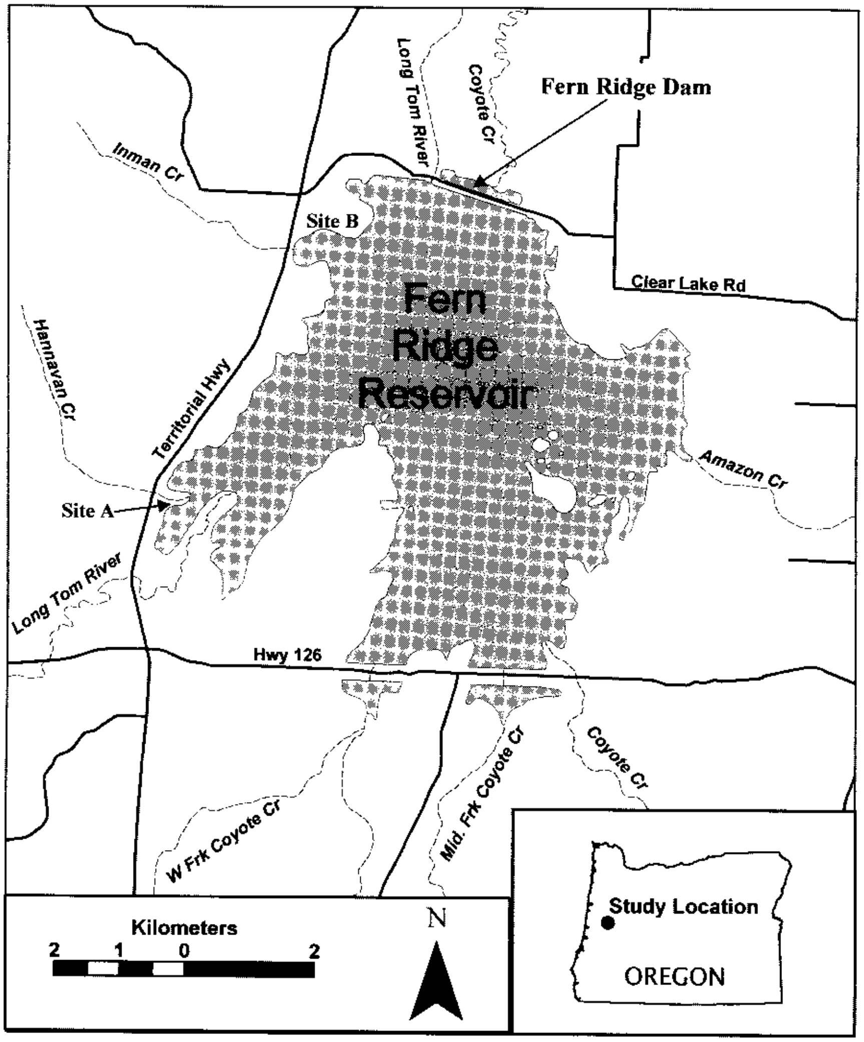

Figure 1. Fern Ridge Reservoir study areas near Eugene, Oregon with South Applegate (Site A) and Tripass (Site B) turtle nesting areas.

poorly drained, this area is less saturated than South Applegate in winter, does not support native wet prairie vegetation, and turtle nests are rarely, if ever, inundated. Turtles are frequently observed basking in the nearby cove. Evidence of turtle movement between the two sites is based upon a male turtle marked on 12 April 1996 near South Applegate that was trapped in Tripass Cove on 22 June 2000 (a distance of 4.95 km). Radio-telemetry studies of western pond turtles in western Oregon reservoirs show that trips of several kilometers along reservoir shorelines are not uncommon (Beal 1993).

Methods

Field Methods

Nest areas were searched daily between 1 June and 25 July 1997, and turtles nested between 4 June and 18 July. We marked 17 nests near Fern Ridge Reservoir and these were incubated in the wild. We excavated the nests on 2 October to determine egg survivorship. Most eggs had not hatched but appeared viable. Usually, successful nests have hatched by the first week of October. The unhatched eggs were taken to an incubator at E. E. Wilson Wildlife Management Area near Corvallis, Oregon, with the hatchlings scheduled for later release as part of an experimental Head Start Program. Most clutches (14 of 17) chosen for removal had at least one intact egg that did not hatch in the incubator, and one sample egg from each of these clutches was analyzed for contaminants. The three clutches without an intact egg available for analysis included two that were successful (hatched 2 from South Applegate and hatched 9 from Tripass), and one that failed (South Applegate). The clutch sizes were 6, 11, and 9. The sample egg technique was used to evaluate residues in a sample egg in relation to hatchability of the remaining eggs in the clutch. To use the approach to evaluate critical residue concentrations, we assume that egg residue concentrations are consistent in eggs within a clutch, which has been shown for bird eggs (Blus 1984).

Analytical Chemistry

Egg contents were weighed and placed in chemically cleaned jars. We sent egg contents to the Great Lakes Institute of Environmental Research at the University of Windsor, Windsor, Ontario, for chemical analyses. Organic chemical analyses were conducted using standard methods (Great Lakes Institute of Environmental Research 1995) to identify 20 OC pesticides and 42 PCB congeners. Data were quantified by comparing sample-peak area against standard-peak area of three standards supplied by the Canadian Wildlife Service. OC pesticides and PCB fractions were analyzed separately on an electron-capture gas chromatograph. The detection limit for OC pesticides and PCBs was 0.1 μg/kg wet weight (ww). OC pesticides and PCBs were confirmed using gas chromatography/mass spectrometry. Methodology for extraction and cleanup was checked by sample blanks, replicate samples, and certified reference samples provided by the Canadian Wildlife Service for OC pesticides and PCBs. Egg volume was determined by water displacement. We converted contents of eggs to an approximate fresh ww (for organics) using egg volume (Stickel et al. 1973). All OC and PCB residues are reported as fresh ww.

Eggs were also analyzed for metals and trace elements (Al, As, Cd, Co, Cr, Cu, Fe, K, Mg, Hg, Mn, Na, Ni, Pb, V, and Zn) by atomic absorption spectrophotometry (Great Lakes Institute of Environmental Research 1995). Dry weight (dw) detection limits (μg/g) were variable and presented with the residue data.

Statistical Analyses

Residue concentrations were presented as geometric means and log-transformed for statistical analyses. The lower quantification limit was halved for samples in which a contaminant was not detected. This value was used to calculate geometric means when > 50% of the samples contained detectable residues. Because of unequal sample sizes, the General Linear Models Procedure (SAS Institute 1996) for analysis of variance was used. Statistical significance was $P \leq 0.05$.

Results

Organochlorine Pesticides and PCBs in Eggs

Variable concentrations of OC pesticides and their metabolites occurred in turtle eggs (Table 1), with DDE, oxychlordane, and *trans*-nonachlor present in all. The geometric mean for total chlordanes (*trans*-nonachlor, *cis*-nonachlor, oxychlordane, and *cis*-chlordane) was 2.32 µg/kg. Two of the three turtle eggs with the highest mirex concentrations (2.22 and 2.69 µg/kg) also contained the degradation product photomirex (0.20 and 0.30 µg/kg). PCBs measured as Aroclor 1254:1260

TABLE 1. Organochlorine contaminants (µg/kg, wet weight) in 14 western pond turtle eggs collected from Fern Ridge Reservoir, Oregon, 1997.

Category	Geometric Mean	Range	Number of Detections
p,p'-DDE	7.76	3.21-11.0	14
Oxychlordane	1.27	0.50-3.51	14
Trans-nonachlor	0.90	0.27-1.32	14
Mirex	0.36	ND[a]-2.69	13
Dieldrin	0.19	ND-0.38	11
Heptachlor epoxide	0.13	ND-0.34	11
Cis-Nonachlor	0.08	ND-0.18	12
1,2,4,5-TCB	NC[b]	ND-0.36	7
Hexachlorobenzene	NC	ND-0.13	4

Note: Pentachlorobenzene; octachlorostyrene; alpha, beta and gamma hexachlorocyclohexane; 1,2,3,4-TCB (tetrachlorobenzene); *trans*-chlordane; p,p'-DDD; and p,p'-DDT were not detected. Photomirex was detected in 2 eggs (0.20 and 0.30 µg/kg) and *cis*-chlordane in 1 egg (0.09 µg/kg).

[a] ND = Not detected.

[b] NC = Not calculated if 50% or more of samples were below detection limit.

TABLE 2. Total PCBs and PCB congeners (μg/kg, wet weight) in 14 western pond turtle eggs collected from Fern Ridge Reservoir, Oregon, 1997.

IUPAC Numbers	Geometric Mean	Range	Number of Detections	IUPAC Numbers	Geometric Mean	Range	Number of Detections
153	4.96	1.05-10.4	14	52	0.33	ND-0.78	11
138	2.93	0.71-3.86	14	206	0.32	0.10-0.63	14
180	2.90	0.50-6.35	14	195	0.19	ND-0.43	13
118	2.27	0.47-3.99	14	171	0.17	ND-0.25	13
179/190	1.22	0.19-2.82	14	158	0.13	ND-0.23	12
183	0.72	0.19-1.36	14	101	0.12	ND-0.32	10
203	0.58	0.13-1.24	14	146	0.11	ND-0.13	13
99	0.55	0.19-0.75	14	149	0.08	ND-0.25	8
182/187	0.51	0.20-0.69	14	200	NC[b]	ND-0.20	7
194	0.45	0.14-0.98	14	66/95	NC	ND-0.43	4
105	0.42	ND[a]-1.15	13	Σ Congeners	19.8	4.78-37.1	14
201	0.33	0.08-0.55	14	Aroclor 1254:1260	39.6	9.57-52.1	14

Note: IUPAC 31/28, 42, 64, 74, 70, 60, 97, 87, 151, 141, 129, 185, and 174 were not detected.

PCBs only present in one egg (IUPAC 49, 0.26 μg/kg; IUPAC 44, 0.11 μg/kg; IUPAC 110, 0.09 μg/kg; IUPAC 172, 0.07 μg/kg).

[a] ND = Not detected.

[b] NC = Not calculated if 50% or more of samples were below detection limit.

TABLE 3. Trace elements and metals (μg/g, dry weight) in 14 western pond turtle eggs from Fern Ridge Reservoir, Oregon, 1997.

Nest Number	Chromium	Copper	Iron	Potassium	Magnesium	Manganese	Sodium	Nickel	Zinc	Mercury
A-1	7.20	36.2	138	5910	1020	2.28	7040	8.59	58.7	ND[a]
A-2	ND	4.39	62.1	4560	1040	1.45	5310	2.62	52.1	0.26
A-6	3.72	5.26	110	6450	1160	2.51	7300	5.59	67.7	ND
A-7	6.84	5.33	98.8	5960	937	5.25	7310	7.97	43.5	ND
A-8	4.61	7.18	120	6310	1460	2.29	8020	6.11	71.7	0.27
A-9	ND	5.89	72.8	6610	1430	2.15	7470	1.30	72.6	ND
A-10	ND	5.35	57.3	5860	917	0.81	5740	0.70	51.8	ND
A-13	ND	5.34	62.1	5530	850	1.19	5810	1.41	59.9	ND
A-14	4.03	9.46	86.2	9720	1060	3.66	12200	0.60	100	ND
A-15	ND	6.36	60.3	6360	579	1.93	8160	1.48	60.5	ND
A-16	44.9	77.0	325	6360	698	4.71	6930	27.7	74.5	ND
B-2	ND	5.98	57.0	7270	1070	1.18	7700	0.56	45.2	0.35
B-4	ND	6.67	85.3	7650	1240	2.97	10500	1.88	94.5	0.54
B-5	ND	4.86	86.2	5390	981	2.85	8070	1.07	87.5	ND
Geometric Mean	NC[b]	8.05	89.3	6330	1000	2.22	7510	2.41	65.1	NC
Detection Limit	2.00	2.00	1.00	16.0	1.00	0.05	3.00	0.50	0.50	0.25

Note: Cobalt found above detection limit (0.30 μg/g) in only one egg (A-16, 0.60 μg/g). Aluminum (4.00), Arsenic (2.00), Cadmium (0.10), Lead (1.50) and Vanadium (1.50) were not found above the detection limits listed here.

[a] ND = Not detected.

[b] NC = Not calculated if 50% or more of samples were below the detection limit.

were present in all turtle eggs and at higher concentrations (39.6 μg/kg) than any of the OC pesticides (Table 2). The dominant PCBs found in the eggs were International Union of Pure and Applied Chemistry [IUPAC] numbers 153, 138, 180 and 118 (Table 2).

Metals and Trace Elements in Eggs

Of special interest are the nonessential elements cadmium, lead, and mercury, but also chromium which is an essential nutrient in the trivalent form (Cr^{+3}) but toxic in the hexavalent form (Cr^{+6}) (Table 3). Cadmium and lead do not readily accumulate in eggs and were not detected in the eggs collected during this study, whereas mercury was detected in 4 of 14 eggs (Table 3). Chromium (form unknown) was detected in 6 of 14 eggs collected. Interactions among metals are common and may be either beneficial or harmful. For example, A-1 and A-16 with the highest chromium concentrations also contained the highest copper, iron, and nickel. Other eggs with chromium detected (A6, A7, A8, and A14) often had copper, iron, and nickel concentrations above the geometric mean.

Egg Residues and Hatchability

Knowledge of the eggs that hatched in the incubator and in the field provide some insight into possible effects of egg residue concentrations on overall hatchability. Unfortunately, 14 nests is a small number for a rigorous statistical assessment. The success or failure of the 14 individual clutches varied widely, but the OC pesticides, PCBs and most metal concentrations were within a relatively narrow range (Tables 1-3). The success of the 14 nests (at least one egg hatched in 57.1% of the nests, and 21.1% of the eggs hatched) was low (Table 4), but with the three nests added where no eggs were available for analysis, the percentages (based on 17 nests) were slightly higher (58.8% of the nests successful, and 25.2% of eggs hatched). With no significant difference in the geometric means for DDE, total PCBs, oxychlordane, *trans*-nonachlor, *cis*-nonachlor, mirex, heptachlor epoxide, and dieldrin in eggs from nests that were successful (hatched at least one egg) versus eggs from nests that totally failed, and the incidence of mercury and chromium (and the extreme values) higher in the sample eggs representing successful clutches, we found no evidence of adverse effects on hatchability related to 10 contaminants (Table 4). The 7-egg clutch with the highest chromium concentration (44.9 µg/g dw) was successful with one egg hatched.

Discussion

Western pond turtles nesting near Fern Ridge Reservoir were exposed to a series of contaminants as evidenced by residue concentrations found in their eggs. However, we were limited in our ability to evaluate possible effects of these contaminants because eggs from only 14 nests were studied. Reproductive success was generally low in 1997, but no obvious relationship between

TABLE 4. Comparison of residue concentrations and hatchability of eggs (in field and incubator) from western pond turtles collected at Fern Ridge Reservoir, 1997.

Nests (N)	Mean (Range)		Geometric Mean µg/kg wet weight (Extreme)								Occurrence (Extreme µg/g dry weight)	
	Clutch Size	Young Hatched	DDE	Total PCBs[a]	OXY[a]	TRNO[a]	CSNO[a]	Mirex[a]	HE[a]	DIEL[a]	Mercury[b]	Chromium[b]
Failed[c] (6)	7.50 (5-10)	0	7.48 (10.81)	35.22 (52.14)	1.01 (1.56)	0.74 (1.14)	0.07 (0.11)	0.22 (2.22)	0.12 (0.17)	0.17 (0.24)	1 (0.35)	2 (6.84)
Successful[d] (8)	8.00 (5-12)	2.88 (1-8)	7.98 (10.99)	43.32 (51.93)	1.51 (3.51)	1.04 (1.32)	0.08 (0.18)	0.54 (2.69)	0.14 (0.34)	0.21 (0.38)	3 (0.54)	4 (44.9)
Combined[e] (14)	7.79 (5-12)	1.64 (0-8)	7.76	39.64	1.27	0.90	0.08	0.36	0.13	0.19	4	6

Note: No statistically significant difference was found in residue concentrations (geometric means) from eggs collected at failed versus successful nests.

[a] Total PCBs (Aroclor 1254:1260), OXY (oxychlordane), TRNO (*trans*-nonachlor), CSNO (*cis*-nonachlor), HE (heptachlor epoxide), DIEL (dieldrin).

[b] Number eggs with detectable concentrations.

[c] No eggs hatched.

[d] At least one egg hatched.

[e] For 17 nests studied (including 3 nests with no eggs analyzed) mean clutch size 7.94 eggs, mean young hatched 2.00.

residue concentrations and hatchability of eggs was found. Perhaps the most meaningful residue comparisons with our data are comparisons with the large series of snapping turtle eggs collected in 1989-91 from the Great Lakes–St. Lawrence River basin (Bishop et al. 1998), although we must recognize that species sensitivity to contaminants may vary. Eggs were collected at one remote reference site (Lake Sasajewun, Algonquin Provincial Park) and eight other sites in areas of concern because of environmental contamination and habitat loss. Organochlorine pesticides and PCB residue concentrations in the western pond turtle eggs from Oregon were low and similar to the snapping turtle data from the remote site at Algonquin Provincial Park, but much lower than all other sites where contaminant-related problems were encountered (Bishop et al. 1998).

Bonin et al. (1995) reported PCB congener composition between snapping turtles and mudpuppies (*Necturus maculosus*) along the St. Lawrence and Ottawa Rivers. In snapping turtle eggs, three congeners (118, 138 and 153) constituted over half of the total PCB concentration. These congeners were also dominant in mudpuppy tissues, but they represented only one-third of the PCBs. Bishop et al. (1996) reported that snapping turtle and herring gull (*Larus argentatus*) eggs had a similar PCB composition pattern. Western pond turtle eggs at Fern Ridge Reservoir also had the same three congeners constituting a similar 51.4% of the total PCB concentration. Bonin et al. (1995) noted that despite differences in the physiology of endotherms such as birds and ectotherms such as reptiles, their studies suggest that the metabolic capacities for detoxification of chlorinated hydrocarbons are similar.

Few metals and trace elements have been analyzed in turtle eggs. The highest chromium value in this study (44.9 µg/g dw) was considerably higher than the 1.2 µg/g ww (perhaps 4.8-6 µg/g dw) extreme for reptilian eggs in an American alligator (*Alligator mississippiensis*) from Florida (Heinz et al. 1991). Interactions among metals also seem apparent, but are poorly understood. Mercury was significantly higher in eggs of the omnivorous snapping turtle than in the sympatric but predominantly herbivorous red-eared slider (Meyers-Schöne 1989, Meyers-Schöne et al. 1993). The western pond turtle is a dietary generalist feeding mostly on vegetation, invertebrates, and, rarely, vertebrates (small fish, tadpoles) (Bury 1986). Thus, much as in birds, differences in contaminant concentrations in turtles seem to reflect the trophic level of the diet.

Our findings regarding contaminants from this study must be considered preliminary because (1) many contaminants were not evaluated, (2) some metals which do not readily accumulate in eggs (undetected or in low concentrations) may concentrate and cause damage elsewhere in the turtles, and (3) the evaluation of more sensitive endpoints like endocrine function, deformity rates, growth rates, and sex ratios was not an option. Embryonic exposure of low-dose pesticides (chlordane, *trans*-nonachlor, DDE) given to red-eared sliders altered expected sex outcomes and hatchling steroid physiology (Willingham 2001). These changes can certainly affect an organism's fitness and the same contaminants were found in turtle eggs from Oregon. One of the first records of wild birds killed by chlordane occurred near Corvallis, although most chlordane uses were banned by 1980 (Blus et al. 1983). More research on contaminants, patterned after snapping turtle studies in Canada, needs to be conducted on turtles in Oregon and other western areas. Nondestructive sampling techniques, including blood samples and tail clips, have been suggested for sampling threatened reptile species (Hopkins et al. 2001). Field studies of turtles are further limited by a poor understanding of relationships between most contaminants and turtle responses, but increased concern over the status of reptile populations has stimulated the demand for assessments of contaminant effects on populations. We acknowledge, however, that factors other than contaminants may be involved in the population decline.

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