An Assessment of Reproductive Health and Development of Snapping Turtles (*Chelydra serpentina*) from the Walpole Delta in the St. Clair River Area of Concern
June 2013

K.D. Hughes, S.R. de Solla, and P.A. Martin

Abstract

Snapping turtle eggs were collected from the Walpole Delta, an area in the downstream Canadian portion of the St. Clair River Area of Concern (AOC) in 2011, analyzed for contaminants and assessed for evidence of impairment of reproductive health and development. Hatching success of artificially-incubated eggs and hatchling deformities were examined as two reproduction and development endpoints associated with elevated contaminant exposure. Hatching success was high (93.5%) and the frequency of hatchling deformities was low (7.7%) and no significant differences were found for either of these endpoints compared to frequencies found at the reference site. Overall, these endpoints were positive relative to most other Great Lakes sites. While concentrations of most organochlorines (e.g., PCBs) in eggs from the AOC were significantly higher relative to eggs from the inland Tiny Marsh reference site, concentrations were low overall and were not significantly different from eggs collected downstream and outside of the AOC in Lake St. Clair in 2001. Mercury concentrations in AOC eggs were high relative to eggs from other Great Lakes sites from 2001-2004; however, the mean mercury concentration was not significantly different from the Tiny Marsh reference site due to high variability in mercury concentrations evident among AOC collection sites. There was no evidence of temporal declines in organochlorines or mercury in eggs collected from assorted sites in the AOC from 1995 to 2011 largely due to high variability within collection years. Overall, despite the fact that current contaminant burdens in Walpole Delta snapping turtle eggs were generally low compared to other Great Lakes sites, current concentrations of PCBs, DDT, and mercury exceeded tissue residue guidelines and remain of concern to wildlife consumers of eggs.

Introduction

Historical industrial and municipal point sources in the upper reaches of the St. Clair River have contributed significant loadings of pollutants to the river which have been detected in downstream regions of the river as well as in Lake St. Clair (OMOEE and MDNR 1995). Contaminants of concern associated with these sources were identified which included hexachlorobenzene (HCB), octachlorostyrene (OCS), polychlorinated biphenyls (PCBs), and toxic metals such as mercury. Historical agricultural practices in this primarily agricultural watershed have also introduced pesticides such as DDT to the waterway which have further impacted water and sediment quality in the St. Clair River. Once introduced into the aquatic environment, many of these compounds become available to biota, bioaccumulate in aquatic organisms and fish and then biomagnify through the food web. Numerous exceedences of water, sediment and fish consumption guidelines were identified in the 1970/80s which in part contributed to the designation of the St. Clair River as a Great Lakes Area of Concern (AOC) in 1985 (OMOEE and MDNR 1991). Since that time, restrictions on discharges, improved industrial and municipal practices as well as sediment remediation projects have reduced contaminant loadings to the

river (Mayne 2008). Significant temporal declines in concentrations of many compounds have been reported in fish species in the St. Clair River and, more consistently, downstream in Lake St. Clair (where extensive fish collection data are available) providing evidence of the success of various management actions employed in the AOC (Gewurtz *et al.* 2010). To date however, few studies have examined effects associated with elevated contaminant exposure in top predator aquatic wildlife species within the Canadian boundary of the St. Clair River AOC.

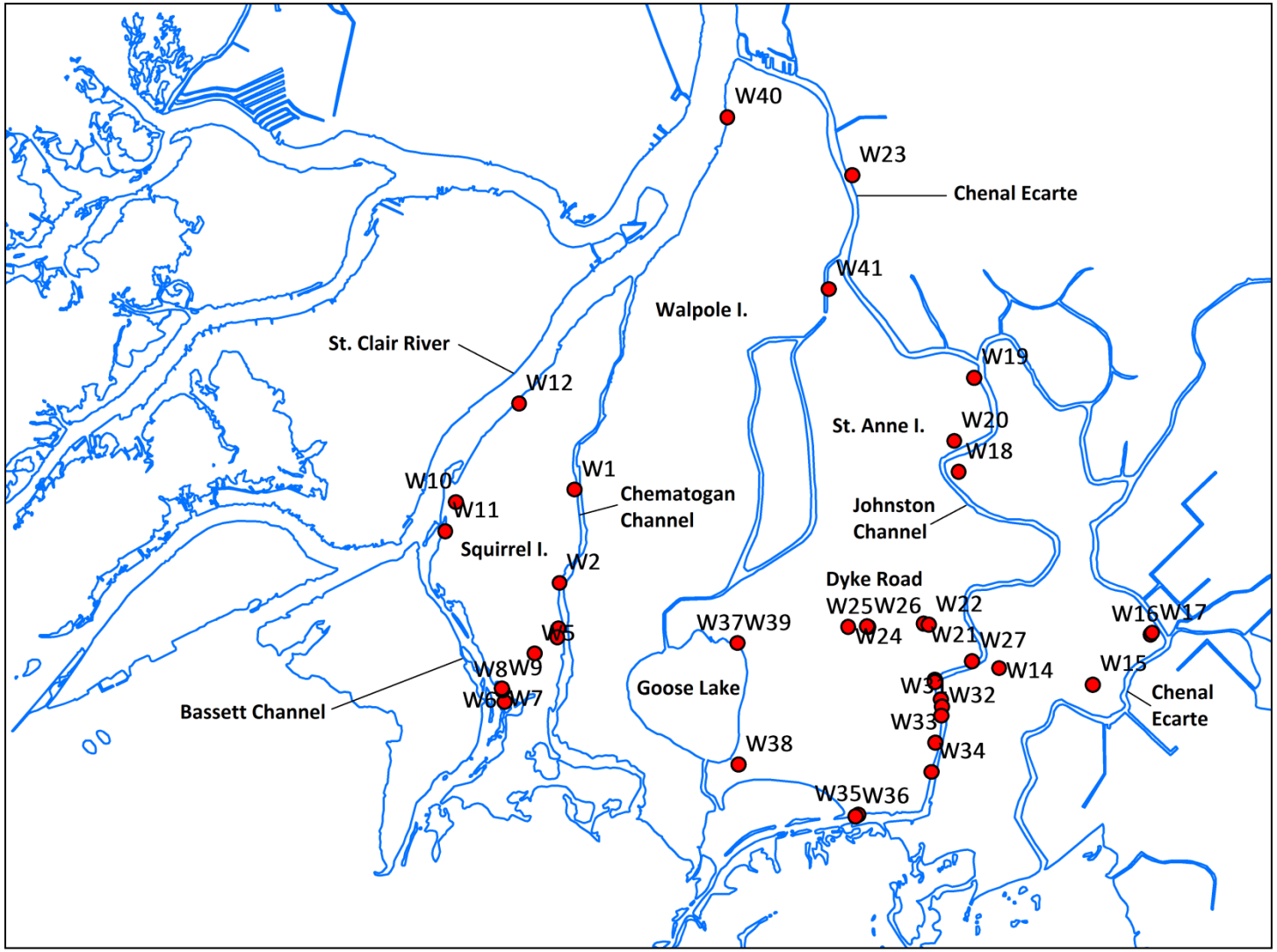
The common snapping turtle (*Chelydra s. serpentina*) has been frequently used as a bio-indicator of contaminants and their effects on wildlife health in Great Lakes AOCs (de Solla *et al.* 2007, 2008). This top predator is a long-lived reptile with a predominantly fish-based diet and a wide geographic distribution where it commonly inhabits most Great Lakes shorelines. With a relatively small home range, this species is also a useful indicator of local sources of contaminants in the aquatic environment (Bishop *et al.* 1998; de Solla *et al.* 2007). Contaminant concentrations in eggs are reflective of maternal contaminant body burdens and increased concentrations of some compounds in eggs have been associated with poor developmental success (e.g., Bishop *et al.* 1991, 1998; Pagano *et al.* 1999; Hopkins *et al.* 2013). In this study, clutches of snapping turtle eggs were collected from multiple locations in the downstream Canadian portion of the St. Clair River AOC (Walpole Delta), artificially incubated in the laboratory and assessed for hatching success and congenital deformities in hatchlings. Eggs were analyzed for contaminants including organochlorines and mercury to assess burdens. In combination with other recent studies of northern leopard frogs (*Rana pipiens*) and waterfowl in the St. Clair River AOC by Environment Canada, the findings of this study using the snapping turtle will be used to assess the current status of the “bird or animal deformities or reproductive problems” beneficial use impairment in aquatic-feeding wildlife in the AOC.

Methods

Snapping turtle eggs were collected from 41 locations in the downstream Canadian portion of the St. Clair River AOC in the Walpole Delta in 2011. Clutches were collected from the three major islands of the delta, Squirrel Island, Walpole Island, and St. Anne Island, which were also proximate to four longest channels of the delta (Bassett Channel, Chematogan Channel, Johnston Channel, and Chenal Ecarte) as well as the lower St. Clair River (Figure 1). Eggs were also collected from a few inland locations at Goose Lake and along Dyke Road on St. Anne Island. For the purpose of this report, three reference non-AOC sites were selected for comparison purposes: 1) Lake St. Clair (consisting of two areas approximately 13 km south of the AOC boundary at Big Point Hunt Club (42° 25' N 82° 24' W) and the St. Clair National Wildlife Area (42° 23' N 82° 25' W), henceforth collectively referred to as “St. Clair NWA”) where eggs were collected in 2001; 2) Tiny Marsh located upstream near Elmvale inland of Lake Huron (44° 36' N 79° 56' W) where eggs were collected in 2001-2003; and 3) Algonquin Provincial Park (46° 36' N 78° 41' W) where eggs were collected in 2001-2004 (for contaminant analysis) and in 2011 (for assessments of hatching success and hatchling deformities).

Entire clutches of eggs were collected within 48 h of oviposition. The eggs from each clutch were placed in plastic containers containing moistened vermiculite, then stored at approximately 18-24^{o}C. Eggs were

Figure 1. Collection locations of snapping turtle clutches in the Walpole Delta in the St. Clair River AOC in 2011. Locations are shown with clutch identifications designated as from W1 through to W41. Four clutches, W13, W14, W15 and W40, were not analyzed for contaminants. Clutch W13 is hidden behind W14.



incubated at the Canada Centre for Inland Waters in Burlington, Ontario. Within two weeks of collection, all eggs were placed in incubators under constant temperature conditions of 27.5 ± 1^{o}C. Water loss through evaporation was replaced every two to three days by spritzing, as required to maintain relatively constant moisture. Eggs were maintained under these conditions until hatching. Hatchling turtles were assessed for gross morphological deformities of the carapacial scutes, eyes, head, limbs and tail.

A subset of eggs, usually five, was selected for contaminant analysis from most clutches; if the clutch was small, fewer eggs were taken for contaminant analysis. Eggs were selected in a pseudo-randomly but stratified manner; eggs were ordered from the egg on top of the nest (last egg laid) to the egg on the bottom of the nest (first egg laid). Each clutch was divided into five groups of approximately equal size, and within each group an egg was selected haphazardly. Subsequently, the egg contents were pooled and frozen in hexane-cleaned amber glass jars at –20^{o}C. A total of 37 clutches of eggs were analyzed for contaminants.

Eggs were shipped frozen to GLIER (Great Lakes Institute of Environmental Research, University of Windsor) for the analysis of PCBs and organochlorine pesticides, which included *p*,*p*'-DDE (a breakdown product of DDT), dieldrin, mirex, sum chlordane (sum of concentrations of oxychlordane, *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor and *trans*-nonachlor), heptachlor epoxide (HE), and hexachlorobenzene (HCB). Octachlorostyrene (OCS) which is not a pesticide but a by-product of industrial processes was also quantified. Quantitative analysis of organochlorine compounds was performed using capillary gas chromatography using a mass selective detector (GC-MSD). Sum PCBs are based on the total sum of 34 individual and co-eluting PCB congeners (IUPAC# 18/17, 31/28, 33/20, 52, 49, 44, 74, 70, 66/95, 101, 99, 87, 110, 118, 105, 151, 149, 153/132, 138, 158, 128, 156/171, 187/182, 183, 177, 180, 191, 170/190, 199, 195/208, 194, 205, 206, and 209). For individual PCB congeners and other organochlorines, the minimum detection limit (MDL) ranged between 0.01 to 0.19 ng/g. For observations below detection limits, maximum likelihood estimation was generally used to calculate replacement values. Using Excel's (Microsoft Corp) iterative Solver function, for each compound observations below the MDL were replaced with values that were fit along a quantile normal plot (log-transformed) of the population mean and variance, which had the maximum log-likelihood (Villanueva 2005). Since each observation is unique, the assumption was made that the replacement value would be proportional to the total contamination. All OCs were positively correlated with *p,p'*-DDE, therefore values below the MDL were sorted such that the replacement values were proportional to *p,p'*-DDE concentrations. For PCBs, it was determined which of PCBs 118, 138 or 153 a particular congener correlated most closely with, and values below MDLs were sorted such that the replacement values were proportional to the concentration of that PCB congener. Organochlorines were expressed on a wet weight basis. Total mercury in eggs was quantified at GLIER using a Direct Mercury Analyzer (DMA-80) based on USEPA Method 7473. Mercury concentrations were determined on a wet weight basis and then converted to dry weight based on percent moisture content. The MDL for mercury was equal to 2 ng/g ww.

In order to assess spatial similarities between inside and outside of the AOC, contaminants, hatching success and hatchling deformity data were statistically compared to data for eggs collected downstream at St. Clair NWA and/or the inland Tiny Marsh reference site where data were available. Data for

Algonquin Provincial Park, an inland site remote from the Great Lakes, are presented for comparative purposes only and were not included in any of the statistical analyses. Since clutches of eggs collected from St. Clair NWA and Tiny Marsh in 2001 and 2002 were analyzed for PCBs and organochlorines using gas chromatography-electron capture detection (GD-ECD), sum PCB concentrations were determined based on the concentrations of the 25 individual PCB congeners common to both GC-ECD and GC-MSD. Concentrations of individual PCB congeners and organochlorines found below the limit of detection in eggs from the reference sites were also estimated using the methods for generating replacement values as reported above. Statistical analyses of organochlorine and mercury data were tested for homogeneity of variances using Levene's test and then $\log_{10}$ transformed (where necessary) to meet conditions of parametric testing. Hatching success and deformities frequencies were calculated for each clutch as proportions of eggs incubated and eggs hatched, respectively, and then arcsine transformed prior to analysis. Since a significant difference in mean percent lipid content was found among eggs from Walpole Delta, St. Clair NWA, and Tiny Marsh ($p=0.012$), organochlorine data were analyzed using an Analysis of Covariance (ANCOVA) using percent lipid as the covariate. When the condition of homogeneity of slopes was met, site effect was examined using an ANCOVA followed by Tukey's HSD test for unequal N to examine significant differences in (unadjusted) means between sites. All results were considered significant at $p<0.05$.

Results

Of all organochlorines in snapping turtle eggs collected from the Walpole Delta in 2011, sum PCBs were found at the highest concentrations with a mean (±SD) of 175.18 (±192.43) ng/g and ranging widely from 7.84 ng/g to 725.73 ng/g in 37 clutches of eggs analyzed (Table 1). Mean concentrations (±SD) of *p,p'*-DDE and sum chlordane were found at the next highest concentrations in eggs and were equal to 9.64 (±12.33) ng/g and 9.22 (±6.32) ng/g, respectively. Dieldrin and octachlorostyrene (OCS) were found at mean concentrations of approximately 2 ng/g while HCB, HE and mirex were equal to approximately 1 ng/g. Maximum concentrations of compounds (**with corresponding clutch id numbers, see Fig. 1**) and nearby waterways were as follows: *p,p*'-DDE and mirex in two clutches collected downstream along Bassett Channel (63.96 ng/g (**W9**) and 2.36 ng/g (**W8**), respectively); sum chlordane, HCB, and HE in one clutch along the St. Clair River just upstream of Bassett Island (28.05 ng/g, 7.59 ng/g, and 2.81 ng/g (**W10**), respectively); sum PCBs and OCS in two clutches downstream along the Chematogan Channel (725.73 ng/g (**W2**)and 10.99 ng/g (**W5**), respectively); and dieldrin in one clutch along the waterway leading into Goose Lake (8.61 ng/g (**W41**)). Generally, the highest concentrations of organochlorines were found in eggs collected along Bassett Channel, downstream along Chematogan Channel, inland of Johnston Channel along Dyke Road and at the mouth of Goose Lake.

Significant differences in mean concentrations of sum PCBs, sum chlordane, dieldrin, OCS and mirex were found among eggs collected from the three study sites: Walpole Delta, St. Clair NWA and Tiny Marsh (Table 1). For these five compounds, concentrations in Walpole Delta eggs were not significantly different from eggs from the downstream St. Clair NWA site but were significantly higher than eggs from the inland Tiny Marsh reference site. No significant differences were found for *p,p'*-DDE and total DDT (data not shown). For HE, the mean concentration was significantly higher in eggs from Walpole Delta

Table 1. Mean concentrations of organochlorines (SD, ng/g, wet weight) in snapping turtle eggs from Walpole Delta in the St. Clair River AOC in 2011 and St. Clair National Wildlife Area (consisting of two sites on Lake St. Clair), Tiny Marsh and Algonquin Park in 2001-2004. Statistical analyses did not include Algonquin Park. Means sharing the same uppercase letter are not significantly different.

Year (s)	Site	N	Sum PCBs	*p,p'*-DDE	Sum Chlordane	Dieldrin	OCS	HCB*	HE**	Mirex
2011	Walpole Delta	37	175.18 (192.43) **A**	9.64 (12.33)	9.22 (6.32) **A**	2.58 (1.90) **A**	2.24 (2.83) **A**	1.37 (1.63) **A**	0.75 (0.81) **A**	0.67 (0.66) **A**
2001	St. Clair NWA	6	101.02 (124.26) **AB**	5.70 (1.52)	4.43 (3.58) **AB**	1.47 (0.99) **AB**	0.43 (0.40) **AB**	0.60 (0.61) **A**	-	0.24 (0.31) **AB**
2001-2003	Tiny Marsh	13	29.28 (34.18) **B**	3.96 (2.21)	1.70 (1.17) **B**	0.42 (0.43) **B**	0.007 (0.006) **B**	0.14 (0.18) **B**	0.14 (0.22) **B**	0.88 (1.97) **B**
2001-2004	Algonquin	14	14.60 (10.46)	3.93 (3.96)	1.27 (1.02)	0.17 (0.29)	0.005 (0.005)	0.10 (0.13)	0.19 (0.19)	0.29 (0.39)

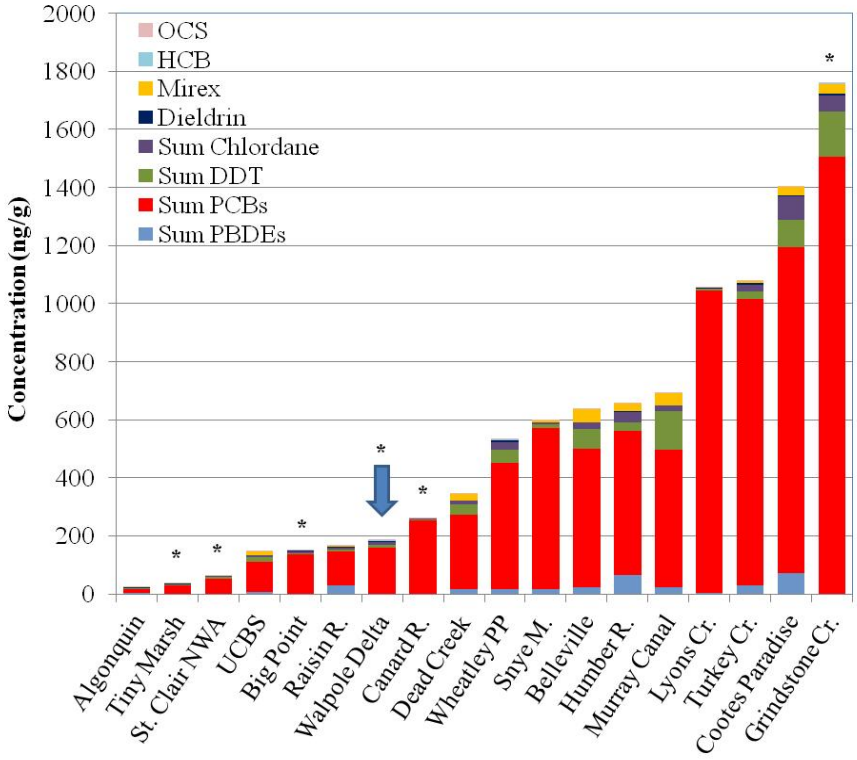
* See text for further discussion following violation of assumption of ANCOVA.

** N values differ for HE analyses only and are equal to 9 and 11 for Tiny Marsh and Algonquin, respectively.

compared to the mean in eggs from Tiny Marsh. For HCB, there was a significant difference in the relationship with percent lipid content among sites which violated an assumption of the ANCOVA (site x % lipid, p=0.007). Following this, a separate slopes ANCOVA was conducted which resulted in a significant site effect (p=0.0001) as well as a significant site by percent lipid effect (p=0.02). Cognizant of the potential for serious bias (i.e., as a result of the significant site by percent lipid effect), Tukey's HSD post-hoc analysis indicated that mean HCB concentrations in eggs from Walpole Delta and St. Clair NWA were similar but both were significantly higher than Tiny Marsh. Mean percent lipid content (±SD) was equal to 4.03 (±1.30)% for Walpole Delta, 4.95 (±0.80)% for St. Clair NWA, and 5.17 (±1.14)% for Tiny Marsh. While not included in the statistical analyses, eggs from Algonquin Park, a remote reference site, generally had the lowest mean concentrations of contaminants.

A Great Lakes perspective of contaminants in snapping turtle eggs represented as cumulative totals of mean sum PCBs, total DDT, sum chlordane and other organochlorines from Walpole Delta in 2011 and other Great Lakes sites from 2001-2004 is provided in Figure 2. Overall, eggs from Walpole Delta were less contaminated relative to most other Great Lakes sites during this earlier time period. Walpole Delta eggs however were more contaminated relative to eggs collected from the two Lake St. Clair sites, Big Point Hunt Club and the St. Clair NWA, situated approximately 13 kilometres south of the St. Clair River AOC border.

Figure 2. Cumulated totals of concentrations of mean sum PCBs, sum PBDEs, sum DDT, and other organochlorines (ng/g, wet weight) in snapping turtle eggs from Walpole Delta in 2011 and various sites in the Great Lakes basin including AOCs, Tiny Marsh and Algonquin Park in 2001-2004. Two sites on Lake St. Clair, St. Clair National Wildlife Area and Big Point Hunt Club, where eggs were collected in 2001 are shown separately. Sites with an asterisk did not have PBDEs measured.



The mean total DDT concentration (±SD), as the sum of *p,p'*-DDE, *p,p'*-DDT and *p,p'*-DDD concentrations, was 10.74 (±13.03) ng/g in clutches of eggs from Walpole Delta. Eleven of 37 clutches (30%) of eggs collected exceeded the total DDT tissue residue guideline of 14.0 ng/g for the protection of wildlife consumers of aquatic biota (CCME 2001). The maximum total DDT concentration was nearly five times this guideline (67.77 ng/g (**W9**)) in a clutch of eggs collected from downstream in Bassett Channel. Using known concentrations for three dioxin-like PCBs which were quantified in this study (mono-*ortho* PCBs #105, 118 and 156), the mean TEQ (±SD) for sum PCBs in Walpole Delta eggs was 0.98 ±1.05 ng/kg which exceeded the TEQ guideline of 0.79 ng/kg for sum PCBs associated with the protection of wildlife consumers of aquatic biota (based on TEFs derived for birds from van den Berg *et al.* 1998; CCME 2001). Specifically, 14 of 37 clutches (38%) exceeded this guideline and of these, nine clutches also exceeded the total DDT tissue residue guideline. Concentrations of other dioxin-like PCBs, dioxins and furans which also known to contribute to toxicity were not determined in this study. Collection locations within the delta that exceeded respective guidelines included eggs collected along Bassett Channel, downstream along Chematogan Channel, inland of Johnston Channel along Dyke Road and at the mouth of Goose Lake.

Temporal changes in concentrations of organochlorines in snapping turtle eggs collected from assorted sites in the Walpole Delta in 1995, 1999, 2007, and 2011 are shown in Table 2. Mean concentrations of compounds showed little evidence of declines in concentrations across study years with notable high variability generally found among clutches within each study year. Since the number of congeners used to determined sum PCBs varied across years (ranging from 35-59 congeners), comparisons of sum PCBs are based on the 27 PCB congeners common to all years of analyses. Overall, these 27 congeners represented on average of 91.74%, 85.41%, 93.70%, and 94.31% relative to the total sum of PCBs determined in 1995, 1999, 2007 and 2011, respectively. It is also important to note that collection sites and clutch numbers varied across study years. For instance, five clutches of eggs were collected from the western portion of the delta on Squirrel Island and Walpole Island in 1995, ten were collected from assorted sites on Walpole Island only in 1999 and 11 were collected from sites on Squirrel Island and on Walpole Island alongside all surrounding channels in 2007. In 2011, 37 clutches were collected from a relatively wider geographic area which also included the eastern side of St. Anne Island along Chenal Ecarte (Figure 1). Such variability in collection sites among years confounds the ability to accurately assess temporal trends.

Mercury concentrations ranged widely in Walpole Delta eggs from 11.11-1315.24 ng/g with a mean concentration (±SD) of 356.03 (±359.41) ng/g on a dry weight basis (or 90.57±86.95 ng/g, 2.47-298.95 ng/g wet weight basis). The highest mercury concentrations were found in eggs collected from inland of Johnston Channel at Dyke Road (944.99-1315.24 ng/g dw (**W24-W26**)), downstream along Bassett Channel (1096.33 ng/g dw (**W9**)), and at the mouth of Goose Lake (1032.64 ng/g dw (**W38**)). Based on collections of ten clutches of eggs collected from the Tiny Marsh reference site in 2001-2004, the mean mercury concentration (±SD) in eggs was relatively lower at 175.80 (±60.47) ng/g dw (or 31.35±12.29 ng/g ww). However, there was no significant difference in mean mercury concentrations between the two sites largely due to the high variability evident in mercury concentrations in Walpole Delta eggs ($p>0.05$; using dry weight concentrations). This result was also consistent using an ANCOVA with percent

Table 2. Mean concentrations of organochlorines (SD, ranges, ng/g, wet weight) in snapping turtle eggs from sites in Walpole Delta in the St. Clair River AOC from 1995-2011. Sum PCBs are based on the sum of 27 PCB congeners common to all chemical analyses in the four study years. N represents the number of clutches of eggs collected.

Year	N	Sum PCBs	*p,p'*-DDE	Sum Chlordane	Dieldrin	OCS	HCB	HE	Mirex
1995	5	194.31 (117.44) 76.35-346.30	17.00 (16.16) 3.30-43.80	13.09 (5.01) 7.30-20.75	2.14 (0.81) 1.10-3.20	1.95 (2.73) 0.15-6.70	1.46 (1.96) 0.20-4.90	1.32 (0.48) 0.80-1.90	1.00 (0.42) 0.70-1.70
1999	10*	153.49 (93.69) 15.00-282.45	8.75 (5.14) 0.50-20.00	47.53 (122.65) 1.60-395.00	14.81 (38.81) 0.05-125.00	1.47 (1.59) 0.05-5.00	1.05 (1.04) 0.50-3.00	1.56 (3.01) 0.05-10.00	0.96 (1.78) 0.05-6.00
2007	11	142.54 (181.22) 15.43-598.43	12.57 (21.56) 0.70-76.28	6.61 (7.34) 1.87-26.89	1.31 (0.49) 0.78-2.48	1.97 (2.50) 0.03-8.04	1.23 (1.43) 0.26-5.01	0.54 (0.35) 0.23-1.48	0.66 (0.67) 0.11-2.11
2011	37	164.14 (179.62) 7.08-670.95	9.64 (12.33) 0.18-63.96	9.22 (6.32) 0.16-28.05	2.58 (1.90) 0.001-8.61	2.24 (2.83) 0.001-10.99	1.37 (1.64) 0.03-7.59	0.75 (0.81) 0.01-2.81	0.67 (0.66) 0.01-2.36

*Denotes the number of pools of clutches in which a pool includes between 1-4 different clutches of eggs collected from a site.

lipid as a covariate since methylmercury, as a component of total mercury, has some degree of lipophilicity. Mercury concentrations in 12 clutches of eggs from Algonquin Park in 2001-2004 were intermediate between Walpole Delta and Tiny Marsh with a mean (±SD) of 270.96 (±177.46) ng/g dw (or 44.49±26.76 ng/g ww). Overall, mercury concentrations in eggs from the St. Clair River AOC were among the highest relative to mean concentrations in eggs collected from other Great Lakes AOCs from 2001-2004 (Figure 3).

In order to assess potential risk to wildlife consumers due to methylmercury, estimates of methylmercury concentrations were determined using known concentrations of total mercury in snapping turtle eggs from the Walpole Delta. A strong linear relationship was evident between methylmercury (MeHg) and total mercury concentrations (THg, as dry weights) in snapping turtle eggs collected from assorted Great Lakes sites in 2001-2004 (MeHg=0.594 x THg - 0.018; $r=0.92$ $p<0.0001$; N=38; EC unpublished data). Using this relationship and upon conversion of estimated MeHg concentrations in Walpole eggs to wet weight concentrations, 16 of 37 clutches (43%) of eggs collected in 2011 exceeded the methylmercury tissue residue guideline of 33.0 ng/g ww for the protection of wildlife consumers of aquatic biota (CCME 2001). Relative to other CCME guidelines for the protection of wildlife consumers, nine of 16 of these clutches also exceeded the total DDT guideline and 13 of 16 of these clutches also exceeded the TEQ guideline for sum PCBs. Therefore, some clutches had consistently high concentrations of these contaminants which, in combination, could be harmful to wildlife consumers.

Mercury concentrations in snapping turtle eggs collected in 2011 were higher than concentrations found in earlier studies of eggs in the AOC. In 1999, the mercury concentration in one pool of eggs collected from Walpole Delta was 110 ng/g dw (Ashpole *et al.* 2004). In 2007, the mean mercury concentration (±SD) was 261.16 (±154.83) ng/g dw in 11 clutches of eggs collected from the area (range=66.51-596.66 ng/g dw). As mentioned above, an assessment of temporal trends is difficult due to variability in collection sites among study years as well as, in this case, few years of egg collections.

Mean hatching success (±SD) of snapping turtle clutches collected from the Walpole Delta in 2011 was high and equal to 93.45 ± 7.46% (N=41 clutches). No significant difference in mean hatching success was found between clutches from the AOC and the Tiny Marsh reference site in 2002-2004 where mean hatching success was relatively lower and equal to 86.02 ± 20.24% (N=20 clutches; $p>0.05$). Comparable to Walpole Delta eggs, mean hatching success was also high in clutches from the remote Algonquin reference site in 2011 (96.24 ± 2.58%; N=12 clutches) and 2002-2004 (92.65 ± 7.80%, N=31 clutches). Overall, mean hatching success was among the highest in turtles from the delta relative to mean frequencies at other Great Lakes AOC sites where studies were conducted from 2002-2004 (Figure 4; de Solla *et al.* 2008; range in means=71.26-92.84%).

The mean hatchling deformity frequency (±SD) in snapping turtles from the Walpole Delta was equal to 7.71 ± 12.23% (N=41 clutches). Similarly, no significant difference in mean deformity frequencies was found between hatchlings from the AOC and those from Tiny Marsh in 2002-2004 (11.27 ± 12.48%, N=20 clutches; $p>0.5$). Also comparable to Walpole Delta, mean hatchling deformity frequencies were low in clutches from Algonquin Park in 2011 (8.93 ± 8.91%; N=12 clutches) and 2002-2004 (5.28 ± 5.99%, N=31

Figure 3. Mean mercury concentrations (SD, ng/g dry weight) in snapping turtle eggs from Walpole Delta in 2011 in the St. Clair River AOC (dark shaded bar) and various sites in the Great Lakes basin including AOCs as well as Tiny Marsh (reference site) and Algonquin Park in 2001-2004. Means are based on number of clutches collected at each site (range=4-41).

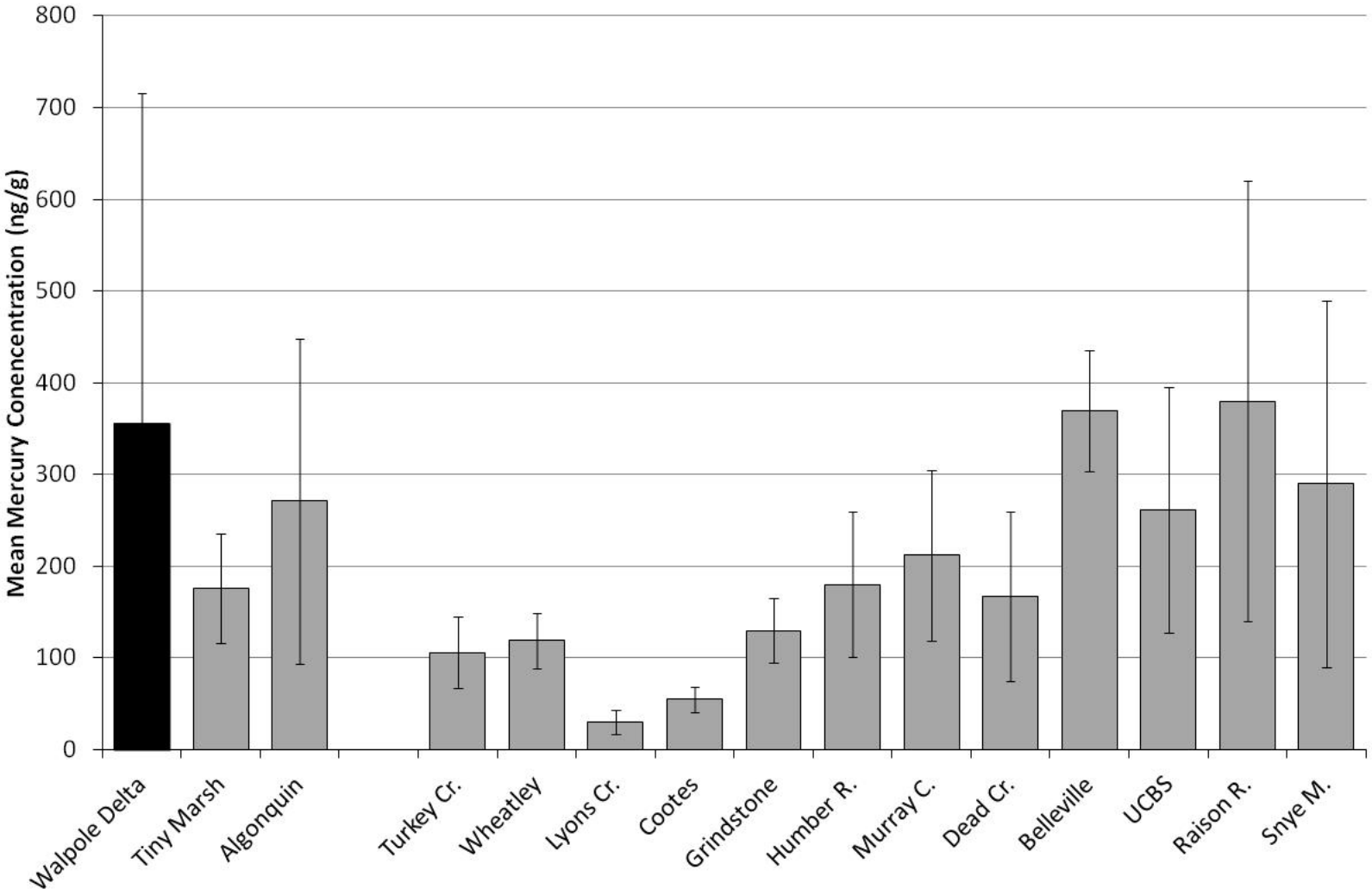
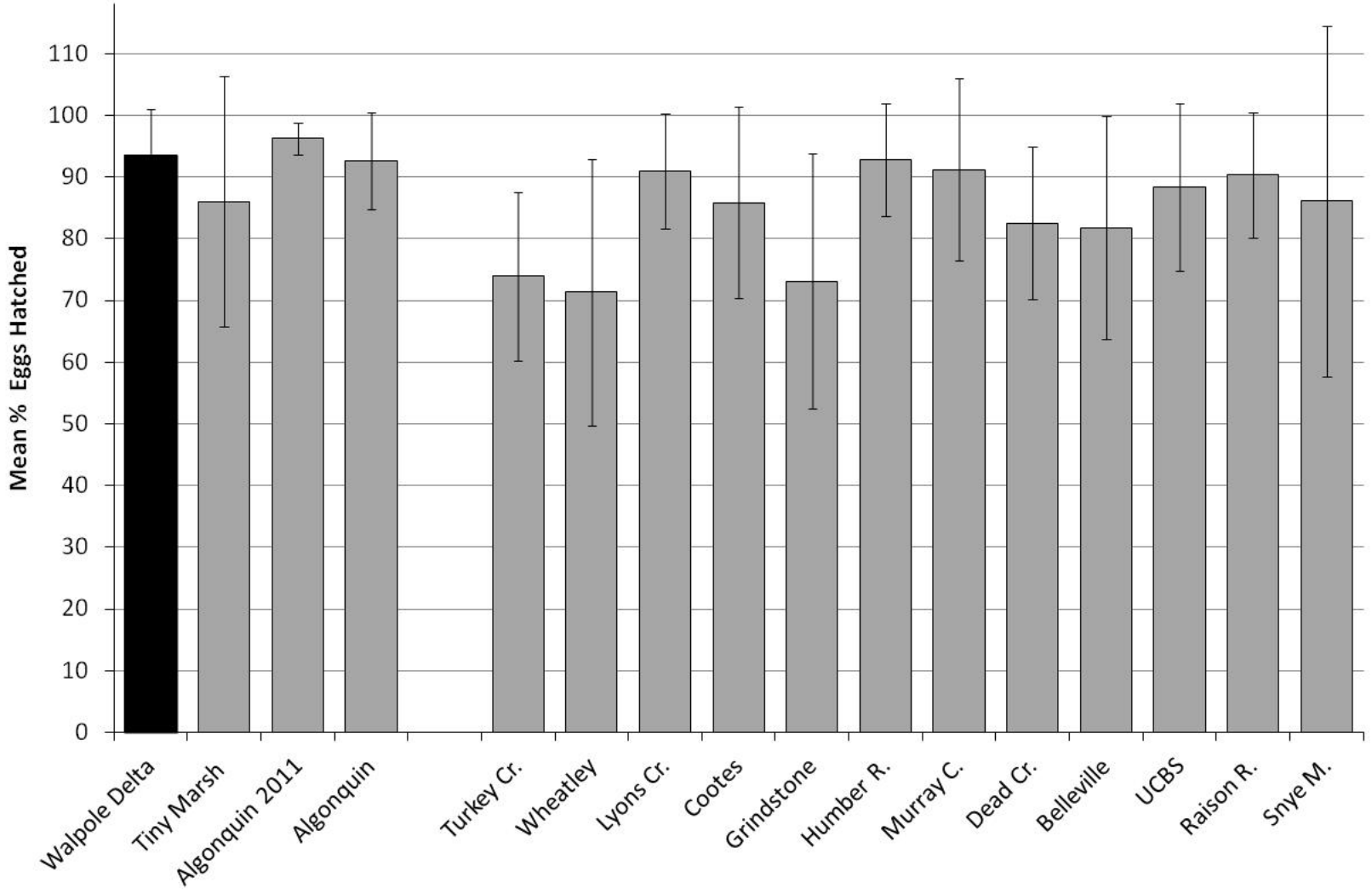


Figure 4. Mean hatching success (SD) of snapping turtle eggs from Walpole Delta in 2011 in the St. Clair River AOC (dark shaded bar), Algonquin Provincial Park in 2011 and various sites in the Great Lakes basin including other AOCs and Tiny Marsh (reference site) in 2002-2004. Means are based on number of clutches collected at each site (range=4-41).



clutches). Mean deformity frequencies at Walpole Delta were among the lowest compared to frequencies found at other Great Lakes AOC sites in 2002-2004 (Figure 5; de Solla *et al.* 2008; range in means=1.29-28.32%). Hatching success and hatchling deformity frequencies were not associated with concentrations of either sum PCBs, total DDT or mercury measured in individual clutches of Walpole Delta eggs ($p>0.05$).

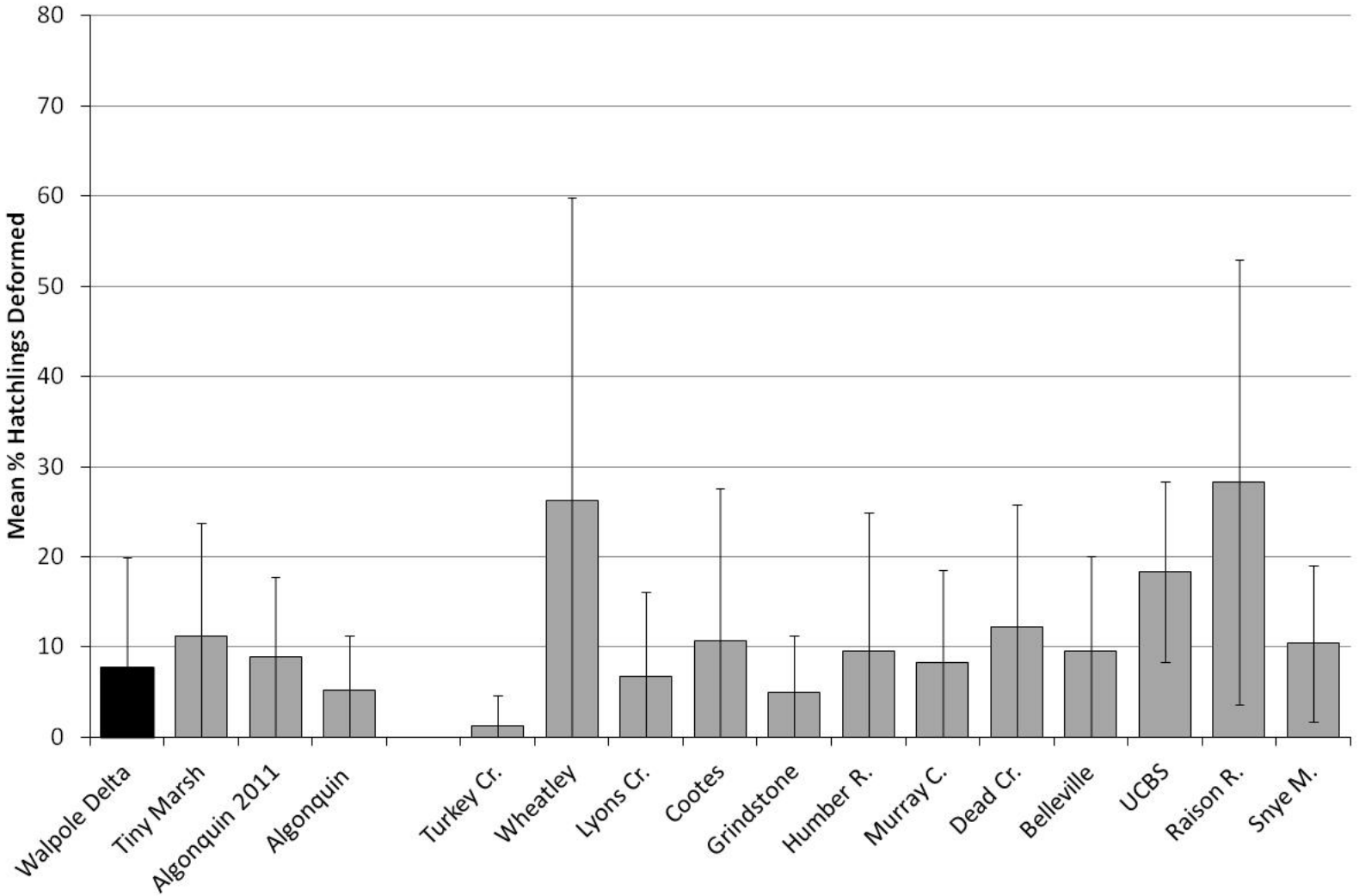
Discussion

Snapping turtle eggs from Walpole Delta in the St. Clair River AOC in 2011 were less contaminated with organochlorines than eggs collected from other Great Lakes AOCs in 2001-2004. This is consistent with earlier studies of contaminants in snapping turtles and mink (*Mustela vison*) from the AOC compared to other AOCs such as Hamilton Harbour during more comparable time periods (Ashpole *et al.* 2004; Martin *et al.* 2006; EC unpublished data). While differences were not significant between sites, eggs from the Walpole Delta in 2011 were 1.7-5.2 times more contaminated relative to eggs collected further downstream at St. Clair NWA for sum PCBs, *p,p'*-DDE, sum chlordane, dieldrin, OCS, HCB, and mirex in 2001 (based on comparisons of means from Table 1). This may be related to the proximity of collection sites relative to upstream St. Clair River contaminant sources and the pattern of water flow as it moves through the Walpole Delta to the centre of Lake St. Clair and to the Detroit River. This was suggested by Gewurtz *et al.* (2010) who reported significant differences in concentrations of mercury, PCBs, OCs, and HCB in juvenile spottail shiner (*Notropis hudsonius*) between the St. Clair River and nearshore areas of Lake St. Clair where water masses from the St. Clair River rarely mix. Overall, areas of elevated exposure to organochlorines in the Walpole Delta appear to be in areas downstream of Bassett Channel and Chematogan Channel, inland of Johnston Channel along Dyke Road and at the mouth of Goose Lake.

High variability in contaminant concentrations in eggs among Walpole Delta sites, as a relatively large study area, suggests that exposure is also highly variable. On a smaller geographic scale, clutches of eggs collected in areas found in close proximity to one another did not necessarily show similar high degrees of contamination. Snapping turtles have very small home ranges and are habitat generalists. Wetlands in the study area are heterogenous, with riverine wetlands, canals that drain agricultural fields, and phragmites (formerly cattail)-dominated marshes often found in close proximity to each other. Although individual snapping turtles often show evidence of specialization for specific habitat types, on a population level all aquatic habitats were equally preferred, at least within one study (Paterson *et al.* 2012). Paterson *et al.* (2012) found that snapping turtles only avoided forest, while bogs, fens, marshes, ponds, creeks, lakes, and rivers were equally preferred. Assuming a similar response of turtles from Walpole Delta, turtles would likely inhabit drainage canals, marshes, and riverine habitats. Variability observed at a smaller geographic scale could be possibly due to turtles using different habitats and ovipositing at the same locality. Variability in contaminant concentrations could also be due to variation in feeding preferences and localized variations in home range contaminant availability (Bishop *et al.* 1994).

Collections of snapping turtle eggs from the Walpole Delta over four study years from 1995 to 2011 show no cursory evidence of temporal declines in concentrations of organochlorines during this period.

Figure 5. Mean deformity frequency (SD) of hatchling snapping turtles from Walpole Delta in 2011 in the St. Clair River AOC (dark shaded bar), Algonquin Provincial Park in 2011 and various sites in the Great Lakes basin including other AOCs and Tiny Marsh (reference site) in 2002-2004. Means are based on number of clutches collected at each site (range=4-41).



Large and dramatic declines in concentrations of many organochlorines have been observed in numerous sport fish from the Great Lakes including Lake St. Clair since the mid-1970s followed by rates of decline which have slowed or stabilized after the early or mid-1990s (Gewurtz *et al.* 2010). Declines in organochlorines were also most pronounced in herring gull eggs from Chantry Island, Lake Huron (upstream) and the Detroit River (downstream) during the mid 1970s to 1990s compared to recent years (EC unpublished data). Similar large declines may have occurred in snapping turtle eggs from the delta however contaminant concentrations in eggs prior to the mid-1990s are lacking so this is speculative. Temporal patterns reported here may be reflective of the period of slowed declines or period of stabilization in concentrations of contaminants since the 1990s. Collections of variable numbers of clutches from assorted sites throughout the Walpole Delta over a relatively short period make an accurate analysis of temporal trends difficult and particularly when such changes during this period may be subtle. For similar reasons, trends in mercury exposure in snapping turtles could not be reliably assessed.

Evidence from this study suggests that wildlife consumers of snapping turtle eggs may be at an increased health risk due to elevated concentrations of DDT, sum PCBs and mercury. Predators of snapping turtles and their eggs or hatchlings include migratory birds and mammals including racoons (*Procyon lotor*), red foxes (*Vulpes fulva*) and mink (COSEWIC 2008). Scientific reports of avian predation upon turtles is rare, but is likely due to a lack of reporting rather than a low frequency of avian predation. In a report about symbiosis between map turtles and grackles, Vogt (1979) reported that "Grackles and Red-winged Blackbirds (*Agelaius phoeniceus*), along with gulls, herons, and crows have been observed consuming hatchling turtles in large numbers as they move from the nests to the water." Mammalian predation, particular raccoons, may be one of the most important factors limiting juvenile turtle survivorship (Browne and Hecnar 2007). Approximately one-third of clutches from Walpole Delta in 2011 exceeded respective guidelines for total DDT, TEQ for sum PCBs, or methylmercury (based on estimated concentrations) associated with the protection of wildlife consumers of aquatic biota and many of these clutches exceeded all three guidelines. In snapping turtle eggs collected from the Walpole Delta in 2007, the guideline for total DDT was also exceeded in approximately one-fifth of clutches (EC unpublished data); exceedences of the TEQ guideline for sum PCBs could not be ascertained following co-elution of additional PCB congeners with two of the three mono-*ortho* PCB congeners (i.e., 105 and 156). The TEQs were likely underestimated, as the majority of TEQs in Great Lakes turtles is due to non-*ortho* PCB 126 (de Solla *et al.* 2001), which was not measured in Walpole Delta eggs. Mean concentrations of other compounds including sum chlordane, HCB, mirex, dieldrin, HE and OCS in snapping turtle eggs were well below fish flesh criteria guidelines for the protection of piscivorous wildlife (Newell *et al.* 1987). Concentrations of OCS most closely approached the wildlife protection guideline with the maximum concentration in one clutch of eggs near Chematogan Channel equal to one-half of the 20 ng/g guideline. Consistent with the pattern for snapping turtle eggs from the Walpole Delta, wildlife protection guidelines were also exceeded for total DDT and PCBs in juvenile spottail shiners collected from sites on the St. Clair River and Lake St. Clair and for total DDT in northern leopard frogs collected along the Johnston Channel (Gewurtz *et al.* 2010; EC unpublished data). HCB concentrations were not notably elevated in Walpole Delta eggs relative to other Great Lakes sites with a mean concentration which was one-half of the highest mean concentration reported at another AOC

(i.e., Grindstone Creek in Hamilton Harbour). Although the Ontario lowest effect level sediment quality guideline was exceeded for HCB in sediment sampled from some Walpole Delta sites as part of an intensive study conducted in 2005 (GLIER and DBS 2008), this was not the case in more recent collections of sediment in 2011 where no exceedences of the 20 ng/g guideline for HCB were found at five sites (EC unpublished data). This included two sites in which HCB concentrations in 2005 had exceeded the provincial sediment quality guideline. These data suggest that current concentrations of DDT, PCBs and mercury in snapping turtle egg are of concern to wildlife consumers while other organochlorines including OCS and HCB may be of less concern in the AOC.

Similar to spatial patterns reported for suspended sediment and juvenile spottail shiners from the St. Clair River AOC (Gewurtz *et al.* 2010; Jia *et al.* 2010), mercury concentrations in snapping turtles from the AOC were also elevated and most notably at sites inland from Johnston Channel at Dyke Road, the mouth of Goose Lake, and downstream at Bassett Channel. The large variability in mercury concentrations in AOC eggs influenced the ability to detect a significant difference with eggs from the Tiny Marsh reference site. Similar to the pattern for organochlorines however, it does suggest that mercury exposure is variable for turtles in the Walpole Delta. Frequent exceedences of the LEL Ontario sediment quality guideline for mercury reported at a number of sites around the Walpole Delta (including Bassett Channel) in 2005 and 2011 (200 ng/g; GLIER and DBS 2008; EC unpublished data) suggests that contaminated sediment in the delta may be a source of mercury to some turtles in the AOC. Mercury concentrations were significantly higher in whole bodies of northern leopard frogs caught along Bassett Channel and near the mouth of Goose Lake compared to other Walpole Delta and AOC sites (EC unpublished data).

While the effects of mercury exposure have been documented in numerous piscivorous fish, bird and mammal species (Scheuhammer *et al.* 2007), little is known with respect to the toxic effects of mercury in reptiles. Hopkins *et al.* (2013) recently demonstrated that total mercury in snapping turtle eggs was negatively correlated with hatching success through increased egg infertility and embryonic mortality. In that study, mercury concentrations in eggs from a mercury-contaminated study site in Virginia were far higher (mean=3000 ng/g dw) than concentrations reported in Walpole Delta eggs (mean=356 ng/g dw) and overall hatching success was also more greatly reduced (mean=80%). Furthermore, while mercury concentrations in some turtle eggs from Virginia exceeded concentrations associated with severe reproductive impairment in other amniotes (birds), the degree of reduced embryonic survival in turtles was not as pronounced might be evident in birds with similar concentrations in eggs. Based on this, they posit that snapping turtles may be more resilient to the adverse developmental effects of mercury exposure than birds. This however is highly dependent on the concentration of methylmercury found in bird eggs compared to turtle eggs which, as a percentage of total mercury, may differ significantly between the two amniotes. The average percentage of methylmercury as total mercury in eggs of 22 bird species was 96% while based on our analysis of methylmercury in eggs of Great Lakes snapping turtles, 47% of total mercury consisted of methylmercury (Ackerman *et al.* 2013; EC unpublished). Therefore, this difference in apparent resiliency may be more a function of the amount of methylmercury deposited into the egg and not due to differences in mercury sensitivity. Using bird thresholds as a surrogate for turtles, the maximum mercury concentration in Walpole Delta turtle eggs

in this study (299 ng/g ww) was well below the predicted threshold of 600 ng/g ww in bird eggs as being protective against adverse reproductive effects for 95% of non-marine avian species (Shore *et al.* 2011). Therefore, while mercury concentrations in Walpole Delta eggs were among the highest compared to turtle eggs from other Great Lakes sites, concentrations may have not been sufficiently elevated to adversely influence reproduction or development. This is supported by the evidence of overall high hatching success and low frequency of hatchling deformities in eggs from the AOC. Little is known however regarding sublethal effects of mercury exposure in reptiles and it is possible that adverse effects associated with increased maternal exposure may be evident at a later time as juveniles grow and develop (as found for PCBs in snapping turtles; Eisenreich *et al.* 2009). Further research is necessary to investigate the effects of mercury on reptiles which, in addition to birds and mammals, serve as important biomonitors of environmental contamination.

Although a commercial ban on harvesting of snapping turtles is in effect in Ontario, snapping turtles can be legally hunted for personal use including consumption. Based on the results of a survey conducted in 1996/97 upstream in the St. Clair River, there is some evidence of human consumption of turtles in the AOC in which 5% of 106 participants reported meals of turtle (species or tissue type not specified; Dawson 2000). With regard to potential human health effects associated with consumption of aquatic wildlife, no guidelines have been developed for consumption of snapping turtles, waterfowl or frogs. Using human consumption guidelines developed for Ontario sport fish (MOE 2011) as a surrogate, total PCBs in over 40% of clutches in 2011 exceeded the first consumption guideline of 105 ng/g for sensitive and general populations and 35% of clutches exceeded the total restriction guideline of 211 ng/g for sensitive populations (i.e. women of child-bearing age and children under 15). No eggs from the St. Clair River AOC exceeded the total restriction guideline of 844 ng/g for the general population. Mercury concentrations exceeded the first consumption guideline of 260 ng/g for sensitive populations in a relatively lower percentage of clutches (i.e., 2.7%). These results support the general recommendation for all of Ontario outlined in the 2011-2012 MOE sport fish guide that the general population avoid consumption of eggs or liver of snapping turtles and sensitive populations avoid eating any part of

Table 3. Summary of exceedences of tissue residue guidelines in 37 clutches of snapping turtle eggs collected from Walpole Delta in 2011. Guidelines for wildlife consumers of aquatic biota (CCME 2001) and Ontario sport fish (MOE 2011) were used to assess protection to wildlife and humans, respectively, following consumption of snapping turtle eggs.

Guideline Type	Contaminant	Tissue Residue Guideline (ww concentration)	% of Exceedences in 37 Clutches of Eggs (Number of Clutches)
Wildlife Consumers of Aquatic Biota	Total DDT	14.0 ng/g	29.7% (11)
	TEQ for Sum PCBs	0.79 ng TEQ/g	37.8% (14)
	Methylmercury	33.0 ng/g	43.2% (16)*
Ontario Sport Fish	PCBs-sensitive populations	105 ng/g (partial restriction)	43.2% (16)
		211 ng/g (total restriction)	35.1% (13)
	PCBs-general population	844 ng/g (total restriction)	0% (0)
	Hg-sensitive populations	260 ng/g (partial restriction)	2.7% (1)

* estimated, based on total mercury concentration

turtles including soups made with meats (MOE 2011). Furthermore it is recommended that in order to reduce risk, muscle should be stripped of all fat prior to consumption. Factors including turtle size (as an indicator of age), tissue type, and amount and frequency of consumption would also influence the degree of exposure and the potential for adverse human health effects. Exceedences of human consumption guidelines for PCBs in common carp (*Cyprinus carpio*) and for mercury in larger-sized walleye (*Sander vitreus*) have also been reported in these species from the St. Clair River and Lake St. Clair (Gewurtz *et al.* 2010). Table 3 provides an overall summary of exceedences of tissue residue guidelines associated with protection to wildlife and human consumers of snapping turtle eggs from Walpole Delta and described throughout this report.

Endpoints associated with reproduction and development have been frequently used to assess the effects of contaminants on the health and survival of wildlife populations (Bishop *et al.* 1998; de Solla *et al.* 2007, 2008; Hopkins *et al.* 2013). While effects on reproductive success and development of snapping turtles have been found in some Canadian Great Lakes AOCs (de Solla *et al.* 2008), these were not evident in snapping turtles from Walpole Delta where hatching success was high (93.5%), hatchling deformity frequency was low (7.7%) and both were statistically similar to the Tiny Marsh reference site. Consistent with this, there was no evidence of impacts on hatching success or elevated deformity frequencies in northern leopard frog embryos exposed to water and sediment from five Walpole Delta sites in 2011 (EC unpublished data). Hepatic sum PCBs in mink trapped on Walpole Island in 2002 were also below laboratory-determined thresholds associated with reproductive effects in mink (Martin *et al.* 2006). Some evidence of impairment of reproductive health and development was found in wild populations of northern leopard frogs from the Walpole Delta in 2006, 2007, and 2011 where an elevated prevalence of deformities in young-of-year frogs and testicular oocytes in male frogs was found at a few survey locations in some years (EC unpublished data). Collections of single clutches from multiple Walpole Delta sites precluded the ability to rigorously examine potential effects at specific locations within the AOC. Overall, contaminant burdens in snapping turtles were low in the AOC relative to studies of other contaminated sites, a finding consistent with the evidence of no impairment for the two reproductive and development endpoints examined in this study. While relatively higher concentrations of mercury in eggs from some sites may not have been sufficiently elevated to influence these endpoints, more subtle effects associated with mercury exposure might have been evident but are unknown at this time. Finally, current concentrations of PCBs, DDT, and mercury exceeded tissue residue guidelines and remain of concern to wildlife consumers of eggs.

Acknowledgments

Our thanks to Kimberley Palonen for technical support and collection and monitoring of eggs in the laboratory as well as calculations of replacement values for contaminant data. Kyna Intini helped in the collection of turtle eggs in the field. We thank Naomi Williams of the Walpole Island Heritage Centre for facilitating and participating in work done within the Walpole Island First Nation and April White of Environment Canada for advice and support throughout. Ontario Ministry of Natural Resources provided scientific collection permits and NWRC/CWS Animal Care Committee provided oversight to the study.

References

Ackerman, J.T., M.P. Herzog, and S.E. Scwarzbach. 2013. Methylmercury is the predominant form of mercury in bird eggs: a synthesis. *Environ. Sci. Technol.* 47: 2052-2060.

Ashpole, S.L., C.A. Bishop, and R.J. Brooks. 2004. Contaminant residues in snapping turtle (*Chelydra s. serpentina*) eggs from the Great Lakes-St. Lawrence River basin (1999 to 2000). *Arch. Environ. Contam. Toxicol*. 47: 240-252.

Bishop, C.A., R.J. Brooks, J.H. Carey, P. Ng, R.J. Norstrom, and D.R.S. Lean. 1991. The case for a cause-effect linkage between environmental contamination and development in eggs of the common snapping turtle (*Chelydra s. serpentina*) from Ontario, Canada. *J. Toxciol Environ. Health*. 33: 521-547.

Bishop, C.A., G.P. Brown, R.J. Brooks, D.R.S. Lean and J.H. Carey. 1994. 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.

Bishop, C.A., P. Ng., K.E. Pettit, S.W. Kennedy, J.J. Stegeman, R.J. Norstrom, and R.J. Brooks. 1998. Environmental contamination and developmental abnormalities in eggs and hatchlings of the common snapping turtle (*Chelydra serpentina serpentina*) from the Great Lakes-St. Lawrence River basin (1989-91). *Environ. Pollut.* 101: 143-156.

Browne, C.L. and S.J. Hecnar. 2007. Species loss and shifting population structure of freshwater turtles despite habitat protection. *Biol. Conserv.* 138: 421-429.

Canadian Council of Ministers of the Environment (CCME). 2001. *Canadian tissue residue guidelines for the protection of wildlife consumers of aquatic biota: summary table*. Updated. In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment, Winnipeg.

COSEWIC. 2008. COSEWIC assessment and status report on the snapping turtle *Chelydra serpentina* in Canada. Committee on the status of endangered wildlife in Canada. Ottawa. vii +47 pp.

Dawson, J. 2000. *Fish stories: a profile of fishing and fish consumption along the St. Clair River area*. Fish and Wildlife Nutrition Project. Health Canada. Ottawa, Ontario, Canada. Great Lakes Health Effects Program Project No. K341813.

de Solla S.R., C.A. Bishop, H. Lickers, and K. Jock. 2001. Organochlorine pesticide, PCB, dibenzodioxin and furan concentrations in common snapping turtle eggs (*Chelydra serpentina serpentina*) in Akwesasne, Mohawk Territory, Ontario, Canada. *Arch. Environ. Contam. Toxicol.* 40: 410-417.

de Solla, S.R., K.J. Fernie, R.J. Letcher, S.G. Chu, K.G. Drouillard, and S. Shahmiri. 2007. Snapping turtles (*Chelydra serpentina*) as bioindicators in Canadian Areas of Concern in the Great Lakes basin. 1. Polybrominated diphenyl ethers, polychlorinated biphenyls, and organochlorine pesticides in eggs. *Environ. Sci. Technol.* 41: 7252-7259.

de Solla, S.R., K.J. Fernie, and S. Ashpole. 2008. Snapping turtles (*Chelydra serpentina*) as bioindicators in Canadian Areas of Concern in the Great Lakes basin. II. Changes in hatching success and hatchling deformities in relation to persistent organic pollutants. *Environ. Pollut.* 153: 529-536.

Eisenreich, K.M., S.M. Kelly, and C.L. Rowe. 2009. Latent mortality of juvenile snapping turtles from the upper Hudson River, New York, exposed maternally and via the diet to polychlorinated biphenyls (PCBs). *Environ. Sci. Technol.* 43: 6052-6057.

Gewurtz, S.B., S.P. Bhavsar, D.A. Jackson, R. Fletcher, E. Awad, R. Moody and E.J. Reiner. 2010. Temporal and spatial trends of organochlorines and mercury in fishes from the St. Clair River/Lake St. Clair corridor, Canada. *J. Great Lakes Res.* 36: 100-112.

Great Lakes Institute for Environmental Research and Department of Biological Services University of Windsor (GLIER & DBS). 2008. *Contaminated sediments in the Walpole Delta: relationship to fish consumption advisories*. Prepared by the Great Lakes Institute for Environmental Research & Department Biological Sciences, University of Windsor for the Great Lakes Sustainability Fund. 87 pp.

Hopkins, B.C., J.D. Willson, and W.A. Hopkins. 2013. Mercury exposure is associated with negative effects on turtle reproduction. *Environ. Sci. Toxicol.* 47: 2416-2422.

Jia, J., L. Thiessen, J. Schachtschneider, J. Waltho, and C. Marvin. 2010. Contaminant trends in suspended sediments in the Detroit River-Lake St. Clair-St. Clair River corridor, 2000-2004. *Water Qual. Res. J. Can*. 45: 69-80.

Martin P.A., T.V. McDaniel, and B. Hunter. 2006. Temporal and spatial trends in chlorinated hydrocarbon concentrations of mink in Canadian lakes Erie and St. Clair. 2006. *Environ. Monitor Assess.*113: 245-263.

Mayne, G. 2008. *St. Clair River Remedial Action Plan Progress Report. Volume 1. Synthesis Report and Environmental Conditions and Implementation Actions (1998-2003)*. 164 pp.

Ministry of the Environment (MOE). 2011. *Guide to Eating Ontario Sport Fish 2011-2012*. Twenty-sixth Edition, revised. Accessed March 28, 2012. http://www.ene.gov.on.ca/environment/en/resources/collection/guide_to_eating_ontario_sport_fish/index.htm

Newell, A.J., D.W. Johnson, and L.K. Allen. 1987. *Niagara River Project: Fish flesh criteria for piscivorous wildlife*. New York State Department of Environmental Conservation. Technical Report 87-3. 145 pp.

OMOEE and MDNR. 1991. *The St. Clair River Area of Concern Environmental Conditions and Problem Definition*. Stage 1 Remedial Action Plan. 466 pp.

OMOEE and MDNR. 1995. *The St. Clair River Area of Concern, water use goals, remedial measures and implementation strategy. Remedial Action Plan Stage 2- recommended plan.* St. Clair River RAP Team. 121 pp.

Pagano, J.J., P.A. Rosenbaum, R.N. Roberts, G.M. Sumner, and L.V. Williamson. 1999. Assessment of maternal contaminant burden by analysis of snapping turtle eggs. *J. Great Lakes Res*. 25(4): 950-961.

Paterson, J.E., B.D. Steinberg, and J.D. Litzgus. 2012. Generally specialized or especially general? Habitat selection by snapping turtles (*Chelydra serpentina*) in central Ontario. *Can J. Zool.* 90: 139-149.

Scheuhammer, A.M., M.W. Meyer, M.B. Sandheinrich, and M.W. Murray. 2007. Effects of environmental methylmercury on the health of wild birds, mammals, and fish. *Ambio* 36: 12-18.

Shore, R.F., M.G. Pereira, L.A. Walker, and D.R. Thompson. 2011. Mercury in nonmarine birds and mammals. In: *Environmental contaminants in biota (2nd edition)*, eds. W.N. Beyer and J.P. Meador, pp. 609-624. CRC Press, Boca Raton, FL.

USEPA Method 7473. Mercury in solids and solutions by thermal decomposition, amalgamation, and atomic absorption spectrophotometry. 17 pp. Accessed December 11 2012. http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/7473.pdf

Van den Berg, M., L. Birnbaum, A.T.C. Bosveld, B. Brunström, P. Cook, M. Feeley, J.P. Giesy, A. Hanberg, R. Hasegawa, S.W. Kennedy, T. Kubiak, J.C. Larsen, F.X.R. van Leeuwen, A.K. Dijen Liem, C. Nolt, R.E. Peterson, L. Poellinger, S. Safe, D. Schrenk, D. Tillitt, M. Tysklind, M. Younes, F. Waern, and T. Zacharewski. 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environ. Health Perspect.* 106: 775–792.

Villanueva, P. 2005. MLE-based procedure for left-censored data excel spreadsheet. Washington, DC: Office of Pesticide Programs. U.S. Environmental Protection Agency.

Vogt, R.C. 1979. Cleaning/feeding symbiosis between grackles (*Quiscalus*: Icteridae) and map turtles (*Graptemys*: Emydidae). *Auk* 96: 608-609.