 

Assessment of the Wildlife Reproduction & Deformities Beneficial Use Impairment in the Hamilton Harbour Area of Concern – Snapping Turtles

Environment and Climate Change Canada – Ecotoxicology & Wildlife Health Division

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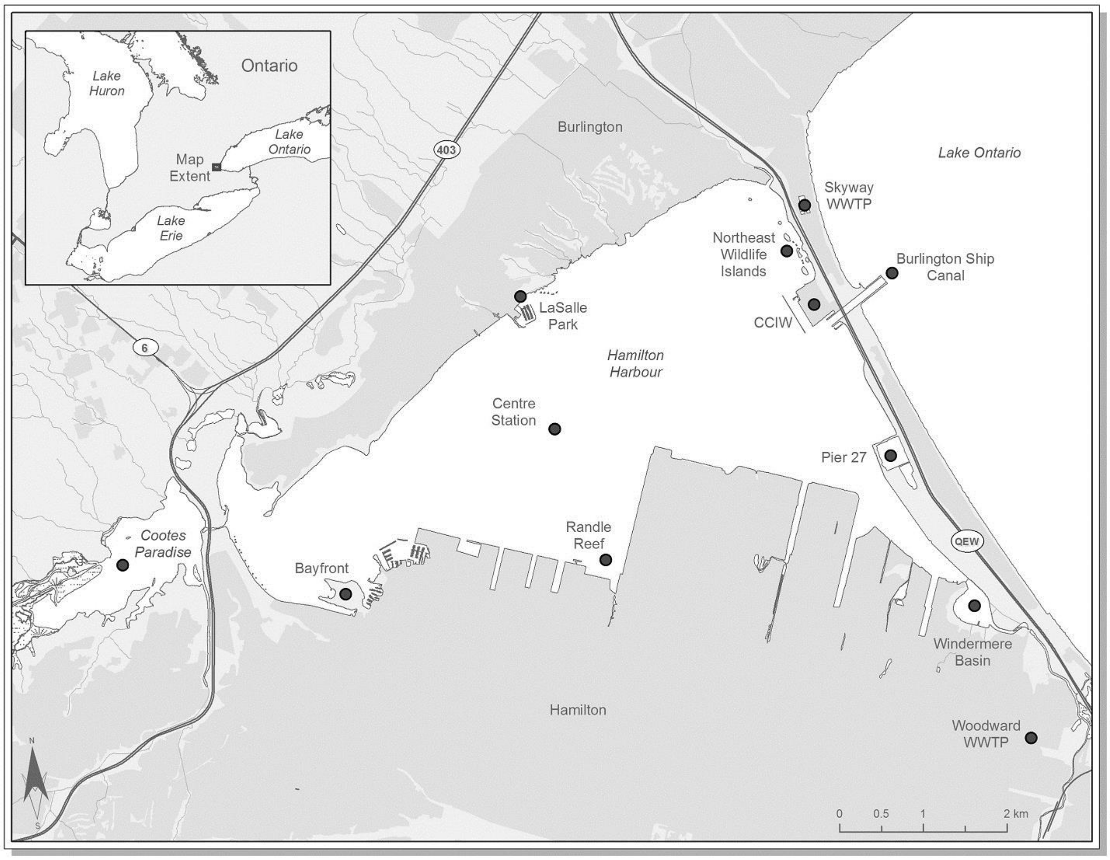
ABSTRACT

Reproduction and development were examined in snapping turtles (*Chelydra serpentina serpentina*) foraging within the Hamilton Harbour Area of Concern in 2012–2014 and 2016. Clutches of freshly-laid eggs were collected from two primary locations within the Area of Concern (AOC) and reference locations outside of the AOC, artificially incubated in the laboratory and assessed for hatching success and hatchling deformities. Several legacy contaminants, metals, and polybrominated diphenyl ethers (PBDEs) were also measured in a subsample of eggs from these clutches in order to examine potential reproductive and development effects associated with exposure to these compounds. In all study years combined, hatching success was over 89% at both Grindstone Creek and Cootes Paradise AOC locations and percentages of deformed hatchlings were less than 11% at the two AOC locations. Hatching success was similar among eggs collected from AOC locations and reference locations in three of four years. In one year (2014), hatching success was significantly lower in clutches from Cootes Paradise compared to the Long Point reference location. After excluding a clutch with abnormally low hatching success, no significant difference in hatching success was found among study locations. Furthermore, reduced hatching success was not found when egg collections targeting this collection area were repeated in 2016. Frequencies of deformed hatchlings were also not elevated in egg collections from AOC locations compared to reference locations in the four study years. Overall organochlorine and PBDE burdens were consistently and significantly higher in snapping turtle eggs collected from Grindstone Creek and Cootes Paradise AOC locations compared to the reference locations. Eggs from these two AOC locations had the highest organochlorine burdens, dominated largely by PCBs, compared to burdens reported in turtle eggs from other Great Lakes AOC sites in both earlier and more recent studies. Several factors relating to both anthropogenic activities and physical attributes of the Harbour likely influenced these spatial patterns which is consistent with the history of contamination in this area. Mercury concentrations in eggs from AOC locations were not consistently different from reference locations and were among the lowest found relative to concentrations in eggs from other Great Lakes collection sites. With the exception of selenium, concentrations of other metals in eggs were largely similar among study locations. Concentrations of PCBs and mercury in turtle eggs in the AOC were below those proposed to have significant adverse effects on reproduction. Large declines in concentrations of sum PCBs and most other organochlorines in eggs since the mid-1980s indicate that exposure to these compounds has decreased in snapping turtles foraging in the AOC. Concentrations of PCBs and total DDT in eggs exceeded tissue residue guidelines for the protection of wildlife consumers of aquatic biota and therefore are of concern to wildlife as consumers of turtle eggs in the AOC. In summary, there was no evidence of impairment for the two reproduction and development endpoints measured in snapping turtles in the Hamilton Harbour AOC that was attributable to local contamination within the AOC.

INTRODUCTION

The Hamilton Harbour Area of Concern (AOC) is one of 43 Great Lakes AOCs that were initially identified by Canada, the United States of America, and the International Joint Commission (IJC) as specific locations where local environmental degradation had severely impaired the area's ability to support aquatic life. Located at the western tip of Lake Ontario, Hamilton Harbour is a 2150 hectare bay that is

Figure 1. Hamilton Harbour Area of Concern (with permission from Hall and O'Connor 2016).



connected to Lake Ontario by a narrow shipping channel. The AOC is comprised of Hamilton Harbour and Cootes Paradise covering an area of approximately 24 km^2 (Figure 1) which drains a large and extensive watershed approximately 500 km^2 in size. Historical discharges of pollutants from local industries and wastewater treatment plants, combined sewer overflows, urban and rural runoff, atmospheric and agricultural loadings, contaminant spills and leachate from landfills impaired water quality and contaminated sediment in the Harbour (Hamilton Harbour Remedial Action Plan 1992). Several contaminants of concern were identified in the AOC including polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and mercury and other metals which contributed to exceedances of water quality objectives, sediment quality guidelines and/or fish consumption guidelines where these were available (Hamilton Harbour Remedial Action Plan 1992).

Fourteen beneficial use impairments (BUIs) were used by Canadian and American federal governments to identify and assess the extent of environmental degradation at Great Lakes AOCs and thereby direct restoration and remediation activities. One of these BUIs, "bird or animal deformities or reproduction problems", addresses contaminant exposure or other anthropogenic environmental stressors on reproductive success or deformity rates in wildlife. Two main species, the herring gull (*Larus argentatus*) and the common snapping turtle (*Chelydra serpentina serpentina*), have been studied extensively in Hamilton Harbour and were identified as sentinel species for assessing wildlife deformities and reproductive problems in the AOC (Bay Area Restoration Council 2004). The common snapping turtle has been frequently used as a bio-indicator of contaminants and their effects on wildlife health in Great Lakes AOCs (Bishop *et al.* 1991, 1998; de Solla *et al.* 2007, 2008). This top predator is a long-lived reptile with a fish-heavy diet and a wide geographic distribution where it commonly inhabits most wetlands in the lower Great Lakes. 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).

Historically, there has been some evidence of reproductive and development problems in snapping turtles in the Hamilton Harbour AOC. In the late 1980s, elevated frequencies of unhatched eggs and hatchling deformities were found in snapping turtles from Cootes Paradise where organochlorine burdens in eggs were also high (Bishop *et al.* 1991). In the mid-1990s, there was evidence of alterations in a sexually dimorphic trait in male and female adult turtles from Cootes Paradise (de Solla *et al.* 1998). This alteration which suggested increased feminization in male turtles was found also in hatchling turtles from Cootes Paradise indicating that this change was likely initiated early in development (de Solla *et al.* 2002). Hatching success of eggs collected from Grindstone Creek which drains into the Harbour was among the lowest of all Great Lakes sites in 2002–2004 and was significantly lower than hatching success of eggs from reference sites (de Solla *et al.* 2008). Consistent with its designation as an AOC, organochlorine burdens, notably PCBs, in snapping turtle eggs from the Hamilton Harbour AOC were elevated relative to other Great Lakes sites in several studies that date back to the 1980s (Struger *et al.* 1993; Bishop *et al.* 1998; Ashphole *et al.* 2004; de Solla *et al.* 2007). In other wildlife, impaired reproduction and development problems were also found in colonial waterbirds from colonies in the AOC in the early 1970s. Low hatchability and gross deformities in chicks of common terns (*Sterna hirundo*) were associated with increased exposure to pollutants in the Harbour during this time period (Connors *et al.* 1975; Gilbertson 1974; Gilbertson *et al.* 1976).

The official status of the wildlife deformities and reproduction BUI in the Hamilton Harbour AOC was changed from “impaired” in 2002 to “requires further assessment” in 2012 following refinements to the delisting objective and time that had lapsed since the last formal assessment (Hamilton Harbour Remedial Action Plan Stakeholder Forum 2012). In 2012, studies of the potential effects of contaminants on reproduction and development in snapping turtles were initiated by Environment and Climate Change Canada (ECCC) to assess the current status of this BUI in the AOC. In this study, clutches of snapping turtle eggs were collected from sites at two general locations in the AOC, artificially incubated in the laboratory and assessed for hatching success and congenital deformities in hatchlings. A subsample of eggs from these clutches were analyzed for legacy contaminants including organochlorines and mercury to assess burdens. The approach used here has been implemented in the St. Clair River (Ontario) and Detroit River (Ontario) AOCs for similar current assessments of this BUI. In combination with other recent studies of herring gulls and northern leopard frogs (*Rana pipiens*) in the Hamilton Harbour AOC by ECCC, 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" BUI impairment in aquatic-feeding wildlife in the AOC.

METHODS

Snapping turtle eggs were collected from two general locations in the Hamilton Harbour AOC at Grindstone Creek and Cootes Paradise in late May or June of 2012, 2013, 2014, and 2016. Specifically, clutches of eggs were collected from four sites around Grindstone Creek at Kicking Horse Trail, Hendrie Park, Laking Garden on Royal Botanical Gardens (RBG) property, and at the mouth of Grindstone Creek from 2012–2014 (Figure 2). Within Cootes Paradise, clutches of eggs were collected from five sites at Community Garden, Hydro One, Spencer Creek, Cootes Drive, and Princess Point from 2012–2014 (Figure 2). An additional year of study was conducted in 2016 when eggs were collected from Cootes Paradise at Princess Point only. Clutches of eggs were also collected from a reference location that was upstream of the Hamilton Harbour AOC consisting of two sites at Beverly Swamp and Valens Conservation Area in 2012 and 2013 and two sites at Freelton and an upstream Spencer Creek site where a single clutch of eggs was collected at each site in 2012 (Figure 3). Following some anomalous hatching success and hatchling deformity results for eggs collected from the upstream reference location in 2012 (see Results), Long Point on the shore of Lake Erie (42.5771°, -80.4468 °) was selected as an alternate reference location for egg collections in 2013, 2014, and 2016.

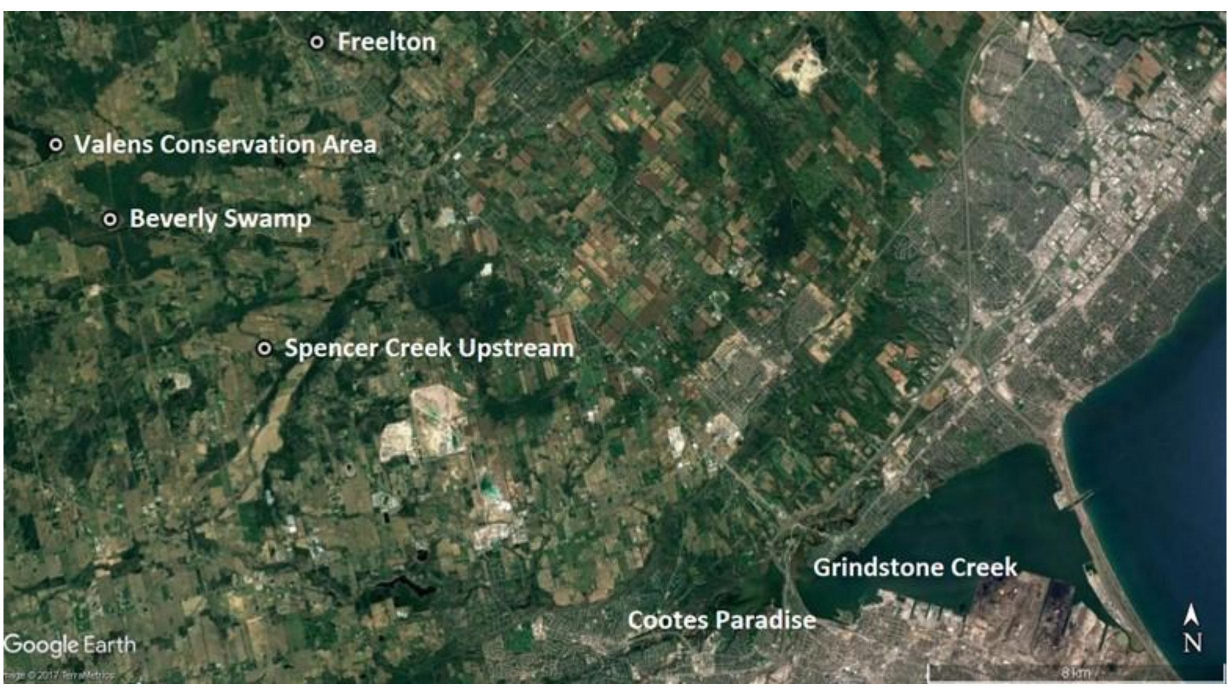
Ten clutches of eggs were collected at each study location in each year with the following exceptions: nine clutches were collected at Grindstone Creek and the upstream reference location in 2012 and six clutches were collected from Cootes Paradise (Princess Point) in 2016. Entire clutches of eggs were collected within 48 h of oviposition. Eggs from each clutch were placed in plastic containers containing moistened vermiculite, moistened with spring water, and then stored at approximately 18–24°C until incubation. Overall, mean numbers ($\pm$SD) of eggs in each clutch over the study were equal to 40.1 $\pm$ 13.6 eggs at AOC locations (N=63 clutches in total) and 30.7 $\pm$ 12.0 eggs at reference locations (N=48 clutches). Typically, a subset of eggs (usually five) was selected from each clutch for contaminant analysis; if the clutch was small, fewer eggs were taken for 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 for chemical analysis. Eggs were sent to one of two laboratories for chemical analysis (see below).

Artificial Incubation of Eggs:

Eggs were 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 24 $\pm$ 1°C and with a relative humidity of 90%. Water loss through evaporation was replaced every two to three days by spritzing as required to maintain relatively constant moisture. Once eggs began to hatch, hatchlings were removed daily, counted and placed in bins containing shallow water. Hatchling turtles were assessed for gross morphological deformities of the carapacial scutes, eyes, head, limbs, toes, and tail. Hatching success and deformities were calculated as the percentage of turtles that hatched or had

Figure 2. Collection sites of snapping turtle clutches at two study locations in Grindstone Creek (comprised of four collection sites) and Cootes Paradise (comprised of five collection sites) in the Hamilton Harbour AOC from 2012–2014 and 2016.

Figure 3. Collection sites of snapping turtle clutches from the upstream reference location (comprised of four sites) in 2012 and 2013. The two study locations at Cootes Paradise and Grindstone Creek in the Hamilton Harbour AOC are shown for reference.



deformities within each clutch. Following the examination of hatchlings for deformities, hatchlings were returned to the water closest to the area where the clutch of eggs had been initially collected. In four cases over the course of the study, clutches that had been partially predated were collected; these clutches were not included in estimates of hatching success and deformity frequencies but were submitted for contaminant analysis.

Contaminant Analyses:

Eggs were chemically analyzed on an individual clutch basis in 2012 and 2013. Egg contents were pooled and frozen in hexane-cleaned amber glass jars at –40°C. In 2014, egg contents from each of ten clutches (based on five eggs per clutch as described above) were combined at each of the study locations to form a single composite pooled sample for chemical analysis. However, following some anomalous hatching success and deformity results for one clutch at Cootes Paradise in 2014 (see Results), eggs were re-analyzed on an individual clutch basis at this AOC location to investigate if these findings were related to chemical burdens in this clutch of eggs. In 2016, due to an oversight, eggs were not immediately taken for contaminant analysis. Instead, whole hatchlings from AOC sites were chemically analyzed for contaminants. At the time of hatching, five individuals were randomly selected per clutch for analysis. Bishop *et al.* (1995) reported that the mean percent difference in the amount of sum PCBs between freshly laid eggs and newly hatched turtles was 12% or less. When reporting on general trends in this report, we will hereafter refer to these hatchlings from 2016 as eggs.

Chemical analyses of snapping turtle tissues for organochlorine compounds and polybrominated diphenyl ethers (PBDEs) were conducted at the National Wildlife Research Centre (NWRC) in Ottawa for egg collections in 2012, 2013, 2014 and 2016 and at the Great Lakes Institute for Environmental Research (GLIER) at the University of Windsor for re-analysis of individual clutches of eggs from Cootes Paradise (10 in total) in 2014. Organochlorine compounds measured included *p,p'*-DDE (dichlorodiphenyldichloroethylene), oxychlordane, *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, hexachlorobenzene (HCB), dieldrin, heptachlor epoxide (HE), mirex, octachlorostyrene (OCS), and polychlorinated biphenyls (PCBs). Sum chlordane is based on the sum concentrations of oxychlordane, *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, and *trans*-nonachlor. Prior to chemical analysis, thawed eggs were homogenized and then underwent neutral extraction and removal of lipids and biogenic compounds by gel permeation chromatography and further clean up by Florisil column chromatography. Quantitative analysis of organochlorine compounds was performed using capillary gas chromatography coupled with a mass selective detector (GC-MSD) operated in selected ion monitoring mode. PBDEs were quantified by GC-MSD operated in the NICI mode at NWRC and by gas chromatography high resolution mass spectrometry methods using a time-of-flight mass spectrometer (GC-MS-TOF) at GLIER. Sum PCBs were based on the sum concentrations of 35–62 individual or co-eluting PCB congeners depending on the year and laboratory performing the analysis. Sum PBDEs were based on the sum concentrations of 15 or 25 individual or co-eluting PBDE congeners at NWRC and GLIER, respectively. Certified internal standards were used for quantification and certified reference materials, blanks and duplicate samples were analyzed for quality assurance purposes. Concentrations of organochlorines and PBDEs are reported in ng/g on a wet weight basis. Method detection limits (MDLs) for organochlorine compounds and individual PBDE congeners ranged from 0.01–5.0 ng/g.

Total mercury was quantified (on a dry weight basis) at NWRC using an Advanced Mercury Analyzer (AMA-254) as described in Laboratory Services methods MET-CHEM-AA-03I and MET-CHEM-AA-03J for eggs collected in 2012 and 2013, respectively. Total mercury was quantified on a DMA-80 as described in MET-CHEM-THg-01A for eggs collected in 2014 and 2016. Eggs collected from Cootes Paradise in 2014 were not re-analyzed on an individual clutch basis for mercury and data are reported for the single pool of eggs analyzed (similar to that reported for Grindstone Creek and Long Point eggs). MDLs for mercury were below 0.006 µg/g in all years. For collections in 2012 and 2013, eggs were also analyzed for manganese, cobalt, nickel, copper, zinc, arsenic, selenium, rubidium, cadmium and lead (on a dry weight basis) by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) on the Perkin Elmer 9000 according to method MET-CHEM-ICP-01A. Using the same instrument, an additional 14 metals that included lithium, beryllium, vanadium, iron, gallium, strontium, molybdenum, silver, tin, antimony, barium, thallium, bismuth, and uranium were screened for in 2013 eggs. Specific to the metals analyzed, Theoretical Detection Limits (TDLs) ranged from 0.02–0.31 µg/g and Practical Detection Limits (PDLs) were five times these concentrations ranging from 0.10–1.57 µg/g. Samples with a metal concentration between the TDL and PDL were flagged as having a detectable concentration of the metal however there was some uncertainly associated with its actual concentration in the sample. Certified reference materials and duplicate samples were also analyzed to ensure correct calibration, accuracy, and reproducibility of test methods. Mercury concentrations in eggs are reported in µg/g both on a dry weight basis and, using percent moisture content, on a wet weight basis for comparisons to published values and thresholds.

Statistical Analysis:

Contaminant burdens and the two biological endpoints were statistically analyzed using a one-way ANOVA for among-location comparisons, which when significant, were followed by Tukey's HSD test or by Student's t-test when there were two locations compared. Data were log-transformed (log_{10}) to meet conditions of equal variance and normality for parametric analysis. If data failed these assumptions, comparisons were made using a Kruskal-Wallis ANOVA followed by a multiple comparison of mean ranks test for all groups or a Mann Whitney test if two locations were compared. For samples that were below MDLs or TDLs, replacement values were calculated for individual PCB congeners, organochlorines and metals using maximum likelihood (de Solla *et al*. 2012). Metal concentrations in eggs were compared statistically on a dry weight basis. A Spearman rank correlation analysis was performed to examine the relationship between mercury and selenium in eggs. All results were considered significant at $p<0.05$.

To assess the potential for adverse effects on wildlife as consumers of turtle eggs in the AOC, concentrations of 2,3,7,8-TCDD toxic equivalents (TEQs) for PCBs, total DDT, and methylmercury in eggs were compared against Canadian tissue residue guidelines developed by the Canadian Council of Ministers of the Environment for the protection of wildlife consumers of aquatic biota (CCME 2001). TEQ concentrations were calculated using toxic equivalency factors developed for both birds and mammals (van den Berg *et al.* 1998, 2006). Total TEQ concentrations in eggs are based on the sum TEQ concentrations of three dioxin-like mono-*ortho* PCB congeners, PCB #105, #118, and #156, measured in eggs from 2012–2014. Total DDT concentrations in eggs are based on the sum concentrations of *p,p'*-DDE, *p,p'*-DDT (dichlorodiphenyltrichloroethane), and *p,p'*-DDD (dichlorodiphenyldichloroethane). Since methylmercury was not quantified in eggs in this study, methylmercury concentrations were estimated based on the strong linear relationship (r^2=0.85) between concentrations of methylmercury and total mercury in an earlier study of snapping turtle eggs collected from assorted Great Lakes sites in 2001–2004 (ECCC unpublished).

RESULTS

Artificial Incubation of Eggs:

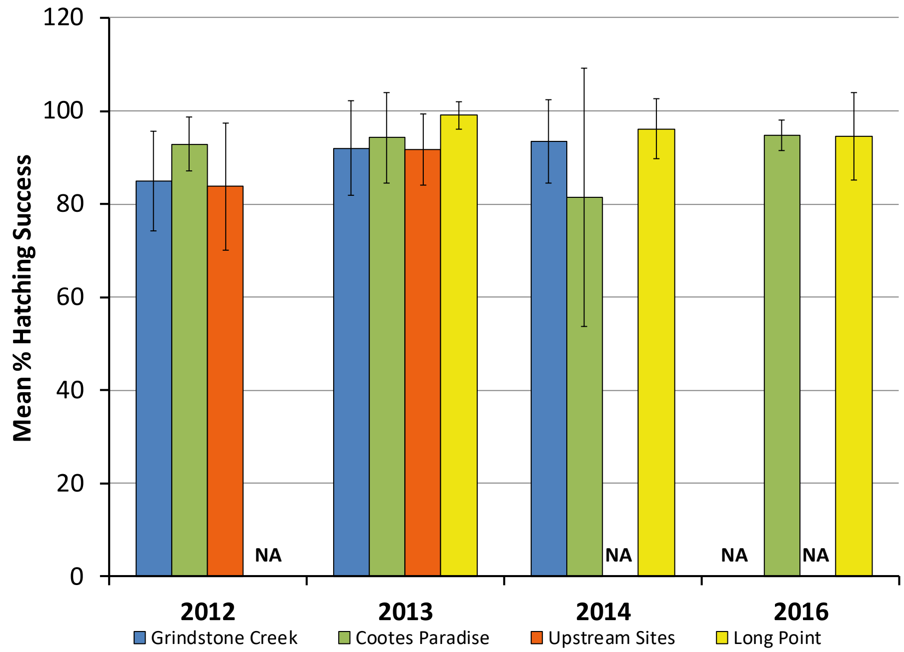
For all years combined, mean hatching success of snapping turtle clutches collected from the two Hamilton Harbour AOC locations was equal to 90.4% at Grindstone Creek (N=27 clutches) and 89.2% at Cootes Paradise (N=35 clutches) from 2012–2016. Mean percentages of deformed hatchlings with at least one deformity, on a per clutch basis, were equal to 11.1% at Grindstone Creek and 9.7% at Cootes Paradise during these years. Spatial trends by year for the two endpoints are shown in Figure 4. No significant differences in hatching success were found among sites in 2012, 2013 or 2016. In 2014, largely influenced by the low hatching success in a single clutch from Princess Point (see details below), hatching success was significantly lower in eggs from Cootes Paradise compared to those from Long Point (*p*=0.04). No significant difference in mean percentages of deformed hatchlings was found among study locations in 2012, 2013, or 2014. In 2016, a significantly greater percentage of deformed hatchlings was found in clutches from Long Point compared to those from Princess Point (*p*=0.04).

Noteworthy are trends for the two endpoints at the reference locations. In 2012, mean hatching success was unexpectedly low (83.7%) and the mean hatchling deformity percentage was notably high (20.0%; N=8 clutches) at the upstream reference site. These values are very different from those found in clutches from Algonquin Provincial Park, another reference location frequently used in Great Lakes studies, where mean hatching success has exceeded 88% and mean percentages of deformed hatchlings were below 9% in similar artificial incubation studies conducted from 2002–2004 and 2011 (ECCC unpublished). Following this finding, an additional reference location on the Great Lakes at Long Point on Lake Erie was added to the study in 2013. Hatching success was relatively higher at the upstream reference location (91.8%) in 2013 compared to that found in 2012, however, the mean percentage of deformed hatchlings and associated variability remained high at 15.9% (N=10 clutches). Given the unexpected and unexplainable findings at the upstream reference location, Long Point was used as the reference location for statistical comparisons with Hamilton Harbour AOC locations in 2013 and in subsequent years. At Long Point in 2013, hatching success of eggs and the percentage of deformed hatchlings, as means, were equal to 99.1% and 4.9%, respectively (N=10 clutches). These values were more consistent with those found at the Algonquin Provincial Park reference location as reported above.

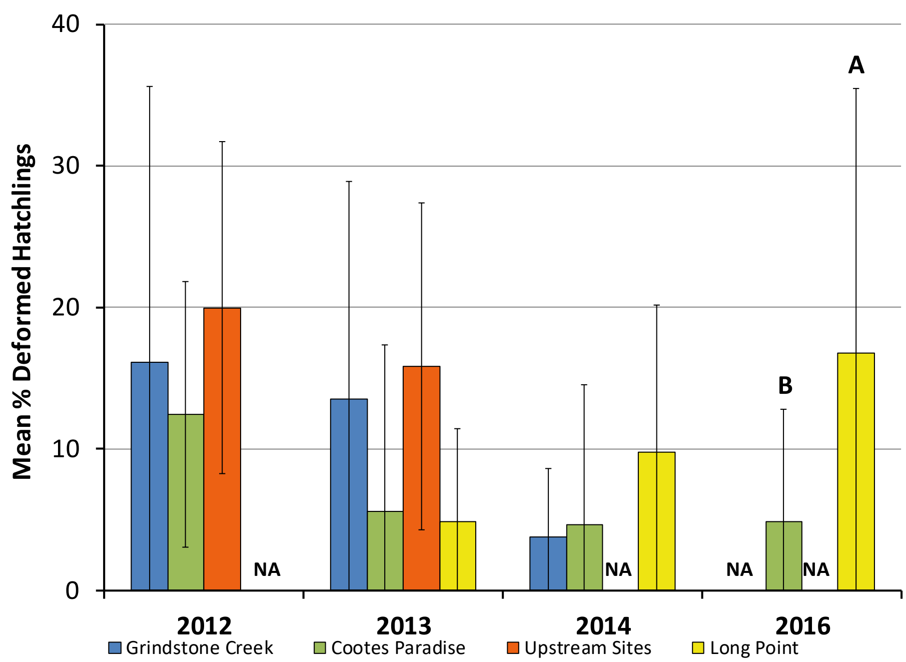
In 2014, hatching success in eggs from Cootes Paradise was significantly lower compared to those from Long Point. High variability among clutches for both endpoints was largely due to one clutch collected from Princess Point that had notably low hatching success (44%, i.e., 22 of 50 eggs hatched) and all

Figure 4. Mean percentages (+SD) of hatching success (a) and deformed hatchlings (b) in clutches of artificially-incubated snapping turtle eggs collected from Grindstone Creek and Cootes Paradise in the Hamilton Harbour AOC and two references locations at Long Point on Lake Erie and sites upstream of the AOC in 2012–2014 and 2016. Different letters indicate significant differences in percentages among study locations within a study year. NA denotes that data are not available. One clutch from Cootes Paradise in 2014 with anomalously low hatching success and high deformities was removed as an outlier (see text for further details).

a)



b)



22 hatchlings (100%) had at least one deformity that included missing or deformed tails and toes. This was the first time in this study that clutches of eggs had been collected from Princess Point for artificial incubation. These surprising results warranted further investigation and subsequent collections of clutches for artificial incubation were conducted at Princess Point in 2016. When this clutch was removed from the analysis, no significant difference in hatching success was found among the three study locations in 2014. Similar to 2013, hatching success in eggs from Long Point was relatively high and the mean percentage of deformed hatchlings was not notably elevated in 2014 with means of 96.1% and 9.8%, respectively (N=10 clutches).

The results of the 2016 collections targeting the Princess Point area were important in demonstrating that the findings of low hatching success and a high percentage of deformed hatchlings from Princess Point in 2014 were not consistent with those observed in 2016 when mean hatching success was nearly 95% and the mean percentage of deformed hatchlings was below 5% (Figure 4).

Contaminant Burdens:

Overall organochlorine and PBDE burdens were consistently higher in snapping turtle eggs collected from Grindstone Creek and Cootes Paradise in the Hamilton Harbour AOC compared to reference locations. In 2012, mean concentrations of sum PCBs, seven other organochlorines, and sum PBDEs were significantly higher in eggs from Grindstone Creek and Cootes Paradise compared to the upstream reference location (Table 1a). This trend was also found in 2013 at AOC locations compared to the Long Point reference location used for statistical comparisons in that study year (Table 1b). The one exception was for dieldrin in 2013 when eggs from Cootes Paradise had similar concentrations to eggs from Long Point. In 2014 when eggs from Grindstone Creek and Long Point were analyzed each as a single pooled sample (and hence no statistical comparisons could be conducted), concentrations of compounds were generally higher at the two AOC locations compared to the Long Point reference location (Table 1c). Following the re-analysis of Cootes Paradise eggs on an individual clutch basis in 2014, there was no evidence that eggs from Princess Point had elevated organochlorine or sum PBDE burdens relative to other clutches that might have explained the low hatching success and high percentage of deformed hatchlings found in that clutch following artificial incubation. Percent lipid content in eggs did not differ significantly among study locations in both 2012 and 2013 and, as such, contaminant burdens were statistically compared on wet weight basis.

In 2016, contaminant burdens in newly hatched turtles from Princess Point were overall lower relative to those of eggs from Cootes Paradise in earlier years (Table 1d). This was likely due to the relatively lower percent lipid content in hatchlings (mean=2.5%) compared to eggs (range in means in Cootes Paradise eggs=5.4%–5.8%). Concentrations were then expressed on a lipid-normalized basis to allow for direct comparisons of concentrations between eggs and hatchlings from Cootes Paradise among study years. On a lipid weight basis, mean concentrations of sum PCBs, *p,p'*-DDE, dieldrin, and OCS in hatchlings were within the ranges of means reported in Cootes Paradise eggs in the previous study years (Appendix A1). For other compounds, mean concentrations in hatchlings were just beyond the range in mean concentrations in eggs. Despite these small differences, lipid-normalized burdens in Cootes Paradise hatchlings were largely comparable to those in eggs. Furthermore, lipid-normalized

Table 1. Mean concentrations of organochlorines and sum PBDEs (SD, ng/g, wet weight) in eggs of snapping turtles from Grindstone Creek and Cootes Paradise in the Hamilton Harbour AOC and two references locations at Long Point on Lake Erie and sites upstream of the AOC in 2012 (a), 2013 (b), and 2014 (c). Mean concentrations of organochlorines and sum PBDEs (SD, ng/g, wet weight) in newly hatched turtles in clutches from Princess Point (Cootes Paradise) in 2016 are shown in (d). Mean lipid percent content (SD, %) is also shown. Different letters indicate significant differences in concentrations among study locations. Sum PCBs are based on the sum concentrations of 35–62 congeners depending on the year of analysis. Sum PBDEs are based on the sum concentrations of 15 or 25 individual or coeluting BDE congeners. N represents the number of clutches of eggs analyzed with the exception of eggs from Grindstone Creek and Long Point in 2014 that were analyzed as a single composite pool. ND indicates that concentrations in all clutches were below the respective method detection limit.

a) 2012 eggs

Location	AOC/ REF	N	% Lipid	Sum PCBs	*p,p′*-DDE	Sum Chlordane	Dieldrin	OCS	HCB	HE	Mirex	Sum PBDEs
Grindstone Creek	AOC	9	5.6 (1.3)	1685 (1121) **A**	81.1 (46.6) **A**	43.7 (30.4) **A**	6.2 (2.0) **A**	1.0 (0.8) **A**	2.2 (1.1) **A**	0.7 (0.3) **A**	30.2 (33.9) **A**	45.8 (21.2) **A**
Cootes Paradise	AOC	10	5.6 (1.2)	1933 (1072) **A**	67.7 (24.8) **A**	74.4 (48.7) **A**	5.3 (2.4) **A**	0.3 (0.2) **A**	1.7 (0.9) **A**	1.0 (0.7) **A**	39.9 (27.0) **A**	52.8 (29.4) **A**
Upstream Sites	REF	9	5.9 (1.8)	10.8 (7.4) **B**	4.4 (3.3) **B**	1.0 (0.8) **B**	1.0 (0.6) **B**	ND **B**	0.3 (0.1) **B**	ND **B**	0.3 (0.2) **B**	0.7 (0.7) **B**

b) 2013 eggs

Location	AOC/ REF	N	% Lipid	Sum PCBs	*p,p′*-DDE	Sum Chlordane	Dieldrin	OCS	HCB	HE	Mirex	Sum PBDEs
Grindstone Creek	AOC	10	5.2 (0.5)	1402 (914) **A**	57.6 (46.7) **A**	35.9 (32.6) **A**	3.5 (2.1) **A**	0.4 (0.5) **A**	1.4 (1.0) **A**	1.1 (0.8) **A**	22.8 (27.1) **A**	44.2 (33.7) **A**
Cootes Paradise	AOC	10	5.8 (1.2)	887 (395) **A**	50.3 (23.2) **A**	37.5 (17.7) **A**	2.3 (1.2) **AB**	0.1 (0.1) **A**	1.0 (0.5) **A**	1.1 (0.6) **A**	14.6 (10.2) **A**	43.1 (30.7) **A**
Long Point	REF	10	5.4 (0.6)	30.8 (34.5) **B**	16.7 (10.6) **B**	3.4 (4.6) **B**	1.1 (0.8) **B**	ND **B**	0.3 (0.1) **B**	0.1 (0.2) **B**	0.2 (0.2) **B**	1.1 (1.0) **B**
Upstream Sites	REF	10	5.6 (1.5)	10.4 (5.5)	4.0 (4.4)	3.4 (5.1)	1.9 (0.5)	ND	0.2 (0.1)	0.02 (0.06)	ND	1.2 (1.3)

c) 2014 eggs

Location	AOC/ REF	N	% Lipid	Sum PCBs	*p,p'*-DDE	Sum Chlordane	Dieldrin	OCS	HCB	HE	Mirex	Sum PBDEs
Grindstone Creek	AOC	1	8.1	1532	99.3	33.1	5.1	0.23	1.0	1.7	19.0	65.9
Cootes Paradise	AOC	10	5.4 (1.3)	1800 (1609)	77.3 (49.2)	53.9 (69.2)	2.6 (2.0)	0.2 (0.1)	0.8 (0.3)	1.0 (1.1)	44.1 (51.8)	61.2 (72.7)
Long Point	REF	1	8.3	106	76.9	13.0	6.5	ND	0.2	1.9	ND	6.5

d) 2016 newly hatched turtles

Location	AOC/ REF	N	% Lipid	Sum PCBs	*p,p'*-DDE	Sum Chlordane	Dieldrin	OCS	HCB	HE	Mirex	Sum PBDEs
Cootes Paradise (Princess Pt.)	AOC	6	2.5 (0.4)	756 (269)	32.4 (13.4)	17.1 (6.3)	1.8 (0.4)	ND	1.2 (0.2)	ND	7.0 (7.3)	28.2 (6.0)

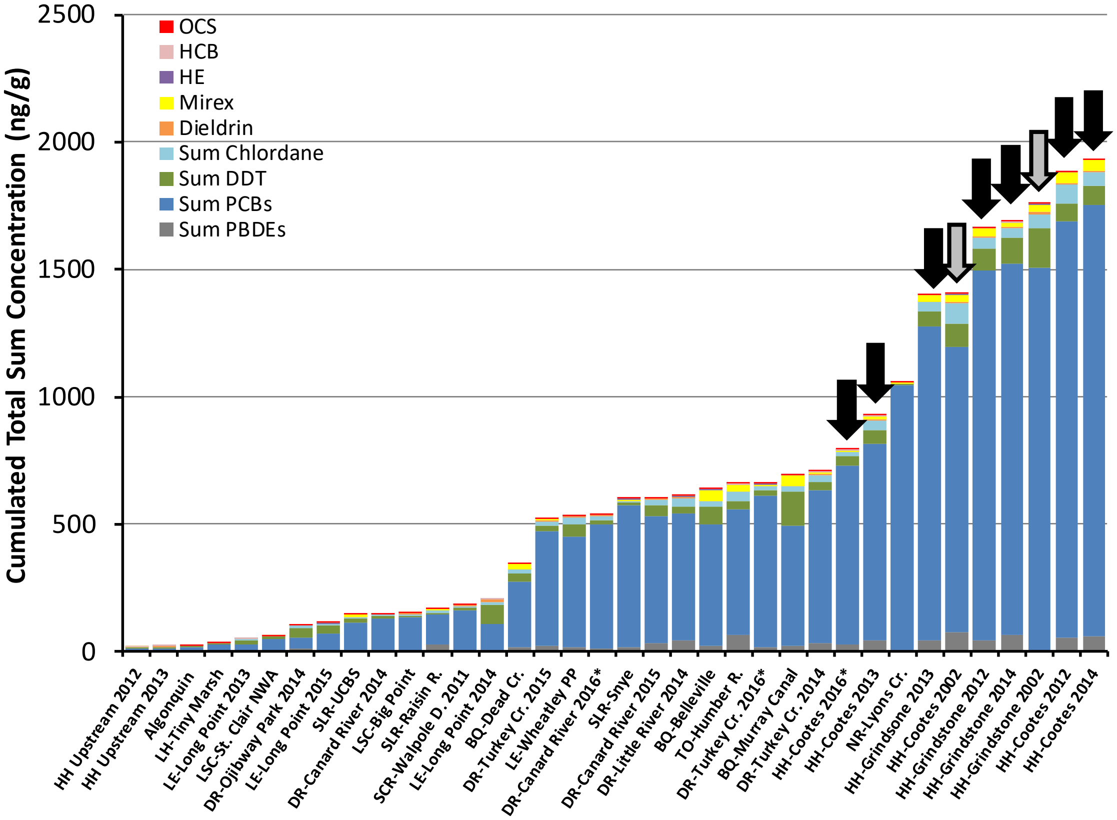
burdens in Cootes Paradise hatchlings also fairly consistently exceeded contaminant burdens in eggs from the two reference locations from 2012–2014 (Appendix A1).

Some consistent trends were found with respect to the relative ranking of contaminants in snapping turtle eggs from AOC locations (Table 1). Of all organochlorines, sum PCBs were found at the highest concentrations with overall means ranging from 887 ng/g in eggs from Cootes Paradise in 2013 to 1933 ng/g in eggs from Cootes Paradise in 2012. Maximum sum PCB concentrations in this study were found in two clutches from Hydro One in 2014 (6092 ng/g) and 2012 (4328 ng/g), one clutch from the mouth of Grindstone Creek in 2012 (3773 ng/g), and one clutch from Hendrie Park in 2013 (3031 ng/g). Concentrations of *p,p′*-DDE ranked second with overall means ranging from 50 ng/g to 100 ng/g in eggs from AOC locations followed by sum PBDEs and sum chlordane which were generally similar (range in means=33–74 ng/g), mirex (range in means=15–44 ng/g), dieldrin (range in means=2–6 ng/g) and lastly, HCB, HE and OCS (all with means below 2.2 ng/g). A similar relative ranking of contaminants was also found in hatchlings from Cootes Paradise in 2016.

Spatial patterns for sum PCBs in turtles in the Hamilton Harbour AOC were examined by grouping clutches by collection site across the four study years using the sum concentration of 35 PCB congeners common to all study sites. In this comparison, eggs from Hydro One and Community Garden were grouped together since collection sites were generally less than 100 metres from each other; the single pool of Grindstone Creek eggs in 2014 was also not included. Mean sum PCB concentrations (wet weight) at eight collections sites ranged from 636 ng/g at Kicking Horse Trail to 1962 ng/g at Community Garden and Hydro One. Overall, sum PCB concentrations varied significantly among all collection sites (p=0.03); however, no significant differences in PCB concentrations were found when individual collection sites were compared. Mean sum PCB concentrations (lipid weight) at collections sites ranged from 13112 ng/g at Kicking Horse Trail to 38947 ng/g at Hendrie Park. There was no significant difference in PCB burdens among collection sites when lipid-normalized PCB concentrations were compared.

A Great Lakes perspective of contaminants in snapping turtle eggs represented as a cumulative total sum concentration of mean sum PCBs, seven organochlorines, and sum PBDEs (where data are available) from Grindstone Creek and Cootes Paradise in 2002 and 2012–2014 and other Great Lakes sites is provided in Figure 5. Total burdens in newly hatched turtles in 2016 are also shown. Overall, eggs from Cootes Paradise and Grindstone Creek were among the most contaminated in studies conducted from 2011–2016 at Great Lakes AOCs that include the St. Clair River (Ontario) and Detroit River (Ontario, study years are indicated) and were at least five times more contaminated that the Long Point and upstream reference locations. Total cumulative burdens in eggs from these two Hamilton Harbour AOC locations in 2002 were also higher relative to other Great Lakes sites at that time (2001–2004).

Figure 5. Cumulated total sum concentration (ng/g, wet weight) of mean sum PCBs, seven organochlorines, and sum PBDEs (where available) in snapping turtle eggs from Grindstone Creek and Cootes Paradise in 2002 (gray arrows) and 2012–2014 (black arrows), associated reference locations, and other sites in the Great Lakes basin including AOCs, Tiny Marsh and Algonquin Provincial Park in 2001–2004. Data for newly hatched turtles in 2016 are also indicated with "*". Data for clutches from Walpole Delta in 2011 in the St. Clair River (SCR) AOC and from sites in the Detroit River (DR) AOC in 2014–2016 are also shown. No PBDE data are available for eggs from Walpole Delta, the two Lake St. Clair sites (LSC) sites, Tiny Marsh, and Grindstone Creek sampled from 2001–2003.



Mercury concentrations in eggs from Grindstone Creek were significantly higher compared to those from the upstream reference location in 2012 and statistically similar to concentrations in eggs from Cootes Paradise (p=0.048; Table 2a). In 2013, mercury concentrations in eggs from Grindstone Creek and Long Point were not significantly different, and both were significantly higher than those from Cootes Paradise (p=0.0002; Table 2b). In 2014, mercury concentrations in the pooled sample of eggs from the two AOC locations were within the range of concentrations reported at these locations in 2012 and 2013 (Table 2c). Mercury burdens in newly hatched turtles from Princess Point in 2016 were also within the range of concentrations found in eggs from Cootes Paradise in previous years. Maximum mercury concentrations (wet weight) in this study were found in one egg clutch from Hendrie Park in 2012 and one clutch from Long Point (pooled sample) in 2014 (both equal to 0.028 µg/g) and in one clutch from Community Garden in Cootes Paradise in 2012 (0.027 µg/g). Concentrations of mercury were found above method detection limits (MDLs) in 100% of egg samples.

Table 2. Mean concentrations (SD, µg/g) of total mercury and selenium in snapping turtle eggs from Grindstone Creek and Cootes Paradise in the Hamilton Harbour AOC and and two references locations at Long Point on Lake Erie and sites upstream of the AOC in 2012 (a), 2013 (b), and 2014 (c). Mean concentrations (SD, µg/g) of total mercury in newly hatched turtles are shown for Cootes Paradise (Princess Point) in 2016 (c). Concentrations are shown as dry weight (dw) and wet weight (ww) concentrations. N represents the number of clutches of eggs analyzed with the exception of eggs collected in 2014 that were analyzed as a single composite pool. Statistical analyses were performed using dry weight concentrations in 2012 and 2013 only. Different letters indicate significant differences in concentrations among study locations.

a) 2012

Location	AOC/ REF	N	Total Mercury dw	Total Mercury ww	Selenium dw	Selenium ww
Grindstone Creek	AOC	9	0.081 (0.033) **A**	0.017 (0.006)	2.2 (0.6) **A**	0.48 (0.13)
Cootes Paradise	AOC	10	0.066 (0.027) **AB**	0.014 (0.007)	1.9 (0.3) **A**	0.41 (0.08)
Upstream Sites	REF	9	0.049 (0.015) **B**	0.010 (0.003)	1.3 (0.2) **B**	0.28 (0.06)

b) 2013

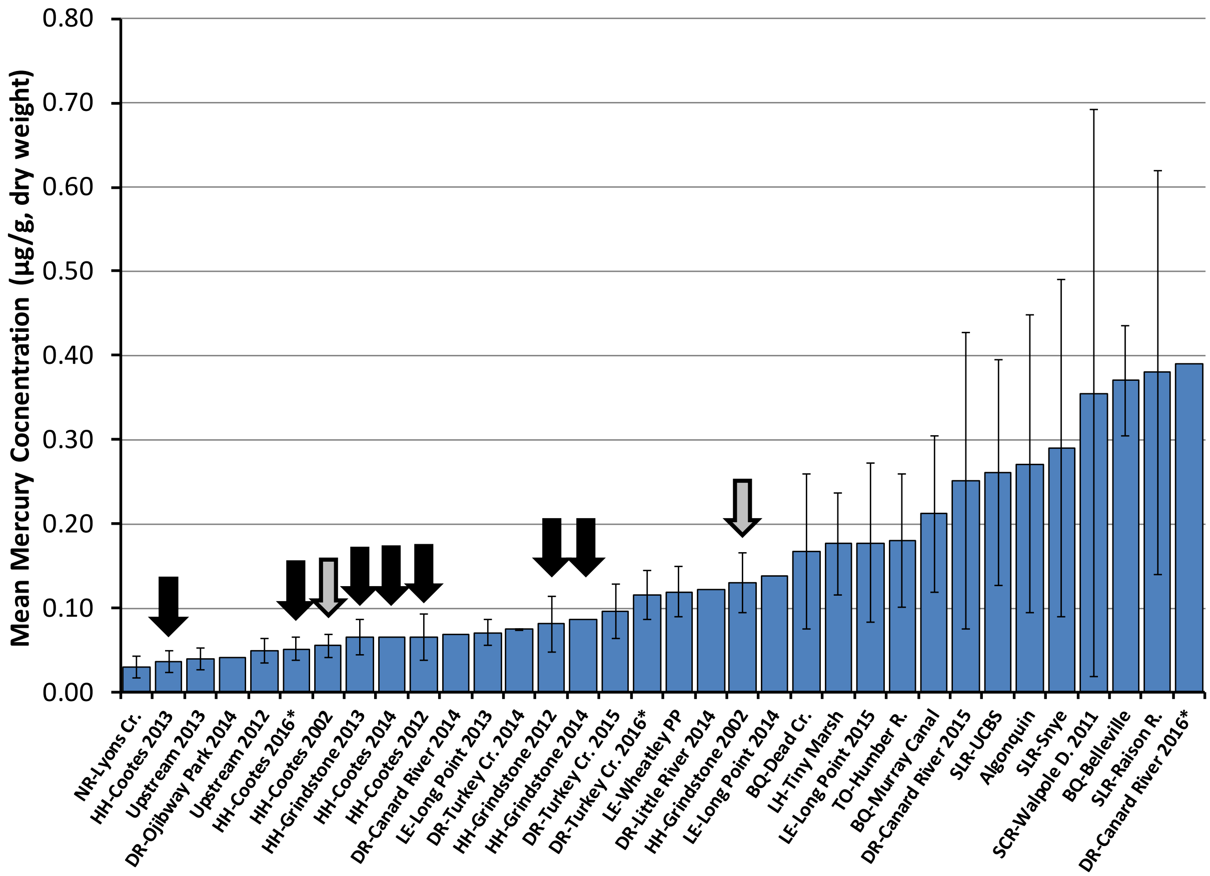
Location	AOC/ REF	N	Total Mercury dw	Total Mercury ww	Selenium dw	Selenium ww
Grindstone Creek	AOC	10	0.065 (0.021) **A**	0.015 (0.005)	2.1 (0.5) **A**	0.47 (0.13)
Cootes Paradise	AOC	10	0.036 (0.013) **B**	0.008 (0.003)	2.0 (0.5) **A**	0.47 (0.16)
Long Point	REF	10	0.071 (0.015) **A**	0.016 (0.004)	1.3 (0.4) **B**	0.30 (0.09)
Upstream Sites	REF	10	0.039 (0.012)	0.010 (0.004)	1.4 (0.4)	0.34 (0.11)

c) 2014 & 2016

Location	AOC/ REF	Year	N	Total Mercury dw	Total Mercury ww
Grindstone Creek	AOC	2014	1	0.086	0.018
Cootes Paradise	AOC	2014	1	0.065	0.015
Cootes Paradise (Princess Pt)	AOC	2016	6	0.051 (0.014)	0.010 (0.003)
Long Point	REF	2014	1	0.137	0.028

Selenium concentrations in eggs from Grindstone Creek and Cootes Paradise were significantly higher than those from the two reference locations in 2012 (p=0.0002; Table 2a) and 2013 (p=0.003; Table 2b). Concentrations of selenium were above probable detection limits (PDLs) in 80 or 100% of eggs from AOC locations and 20-100% of eggs from reference locations in 2012 and 2013 (Appendix A2). Maximum selenium concentrations (wet weight) in this study were found in two egg clutches from Spencer Creek in 2013 (0.78 µg/g and 0.72 µg/g) and in one clutch from Hendrie Park in 2013 (0.71 µg/g). No significant correlation was found between mercury and selenium concentrations in turtle eggs from the two AOC locations and Long Point in 2012 and 2013 (N=49 clutches). However within the AOC, the correlation was highly significant (r_s=0.52, p=0.0007, N=39 clutches). Overall, mercury concentrations in eggs from the two Hamilton Harbour AOC locations in 2012–2014 were among the lowest found relative to concentrations in eggs from other Great Lakes sites (Figure 6).

Figure 6. Mean mercury concentrations (±SD, µg/g, dry weight) in snapping turtle eggs from Grindstone Creek and Cootes Paradise in 2002 (gray arrows) and 2012–2014 (black arrows), associated reference locations, and other sites in the Great Lakes basin including AOCs, Tiny Marsh and Algonquin Provincial Park in 2001–2004. Data for newly hatched turtles in 2016 are also indicated with "*". Data for eggs collected from Walpole Delta in 2011 in the St. Clair River (SCR) AOC and from sites in the Detroit River (DR) AOC in 2014–2016 are also shown.



In 2012, concentrations of copper (Cu), manganese (Mn), nickel (Ni), rubidium (Rb), and zinc (Zn) were found above the PDL in 100% of egg samples (Table 3a). Mean concentrations of these metals were not significantly different among the three study locations. In 2013, a larger number of metals were screened for in eggs. In addition to Cu, Mn, Ni, Rb, and Zn, these included barium (Ba), iron (Fe), gallium (Ga), lithium (Li), strontium (Sr) and vanadium (V) which were found above respective PDLs in at least 70% of egg samples at study locations (Table 3b). Significant variation in concentrations of Li, Mn, Sr, and V were found in eggs among the two AOC locations and Long Point ($F_{2,27}$>6.90, p<0.004). Trace elements including arsenic (As), cobalt (Co), and lead (Pb) analyzed in 2012 and 2013 and molybdenum (Mo) analyzed in 2013 only were found above respective PDLs less frequently, i.e., in less than 30% of egg samples, at study locations (Appendix A2). Cadmium (Cd) was not detected (i.e., concentrations were below the theoretical detection limit) in any egg samples in 2012 and 2013. Beryllium (Be), silver (Ag), tin (Sn), antimony (Sb), thallium (Tl), bismuth (Bi), and uranium (U) were also not detected in any

Table 3. Mean concentrations of metals (SD, µg/g, dry weight) in snapping turtle eggs from Grindstone Creek and Cootes Paradise in the Hamilton Harbour AOC and two references locations at Long Point on Lake Erie and sites upstream of the AOC in 2012 (a) and 2013 (b). N represents the number of clutches of eggs analyzed. Different letters indicate significant differences in concentrations among study locations.

a) 2012

Location	AOC/ REF	N	Cu	Mn	Ni	Rb	Zn
Grindstone Creek	AOC	9	1.8 (0.3)	1.5 (0.4)	0.24 (0.05)	2.9 (1.4)	74.8 (4.8)
Cootes Paradise	AOC	10	2.0 (0.5)	1.3 (0.7)	0.24 (0.04)	3.4 (1.1)	79.7 (9.1)
Upstream Sites	REF	9	1.8 (0.6)	1.6 (0.5)	0.24 (0.02)	2.7 (0.8)	76.9 (7.1)

b) 2013

Location	AOC/ REF	N	Ba	Cu	Fe	Ga	Li	Mn	Ni	Rb	Sr	V	Zn
Grindstone Creek	AOC	10	15.9 (6.5)	2.0 (0.9)	74.3 (9.4)	0.56 (0.23)	0.06 (0.04) **B**	1.9 (0.4) **A**	0.29 (0.06)	3.0 (1.3)	18.4 (9.7) **B**	0.18 (0.03) **AB**	81.5 (7.7)
Cootes Paradise	AOC	10	20.4 (10.8)	2.7 (0.5)	77.6 (9.9)	0.72 (0.38)	0.12 (0.03) **A**	1.3 (0.4) **B**	0.34 (0.12)	3.6 (1.5)	57.2 (35.7) **A**	0.16 (0.01) **B**	73.6 (7.1)
Long Point	REF	10	24.0 (8.6)	2.1 (0.8)	67.1 (9.6)	0.86 (0.30)	0.09 (0.02) **AB**	1.5 (0.4) **B**	0.36 (0.09)	4.3 (2.3)	24.2 (7.7) **B**	0.20 (0.03) **A**	69.5 (15.2)
Upstream Sites	REF	10	24.4 (8.2)	1.4 (0.4)	69.0 (6.8)	0.86 (0.28)	0.10 (0.04)	1.9 (0.7)	0.38 (0.10)	2.9 (0.9)	30.1 (10.3)	0.16 (0.03)	83.0 (7.6)

samples in 2013. Appendix A2 provides a full listing of mean concentrations of metals and percentages of egg samples found above respective PDLs by study location in 2012 and 2013.

Temporal Trends:

Long term changes in exposure to organochlorines in snapping turtles in the AOC were evident in collections of eggs from Grindstone Creek and Cootes Paradise from 1984/86–2014 (for years where data are available; Figure 7). Number of clutches analyzed ranged from 6-15 per year with the exception of collections at Cootes Paradise in 1987, 1991 and 1993 and at Grindstone Creek in 1984 where 1–3 clutches were analyzed; the single pooled sample of 10 clutches is shown for Grindstone Creek in 2014. Regression analysis was performed using mean concentrations for each collection year and data were ln transformed prior to analysis; see Hughes *et al*. 2016 for further details). Overall, concentrations of sum PCBs (based on the sum concentration of common PCB congeners over time), *p,p'*-DDE, sum chlordane, mirex, dieldrin, HCB, and HE declined significantly in turtle eggs from both AOC locations (r^2>0.36; p<0.04; Figure 7). There was no significant change in levels of OCS in eggs from Grindstone Creek from 2002–2014 while concentrations of OCS declined significantly in eggs from Cootes Paradise over a relatively longer time period from 1986–2014 (r^2=0.86, p<0.001). Based on the exponential curves (Figure 7), concentrations of organochlorines appear to have levelled off in more recent years. Decreases in mean concentrations between the first and last year of analysis in eggs from Grindstone Creek ranged from 70.8% for *p,p'*-DDE to 96.6% for HCB. For eggs from Cootes Paradise, decreases in mean concentrations between the first and last year of analysis ranged from 28.7% for mirex to 98.0% for OCS.

For the two metals, no significant changes in concentrations of mercury and selenium were found in eggs from 2002–2014 at both of the AOC locations (Figure 7). This also reflects a relatively shorter time period compared to the legacy organochlorines that were analyzed in eggs for nearly three decades. Similarly, no significant changes in sum PBDE concentrations (based on 8 common congeners among years) were found from 2012–2014 in eggs from Grindstone Creek (range in sum PBDE concentrations=43.9–65.8 ng/g; N=3 years; data not shown) and from Cootes Paradise (range=42.7–73.3 ng/g; N=4 years). Analysis for 2,3,7,8-TCDD in eggs (analyzed as a single pool in each study year) was conducted infrequently during this period and only until 2002. A significant decline in 2,3,7,8-TCDD concentration was found in eggs from 1989–2002 at Cootes Paradise (r^2=0.78, p=0.046, N=5 years) and a 90% decrease in concentration was found in eggs from Grindstone Creek between 1984 (67.0 pg/g) and 2002 (6.4 pg/g).

Figure 7. Temporal trends in mean concentrations of eight organochlorines and two metals in snapping turtle eggs from Grindstone Creek (GC) and Cootes Paradise (CP) in the Hamilton Harbour AOC from 1984/86–2014 (where data are available). Concentrations are shown in ng/g for organochlorines (as wet weights) and in µg/g for mercury (Hg) and selenium (Se, as dry weights). Exponential curves are provided where temporal declines were significant.

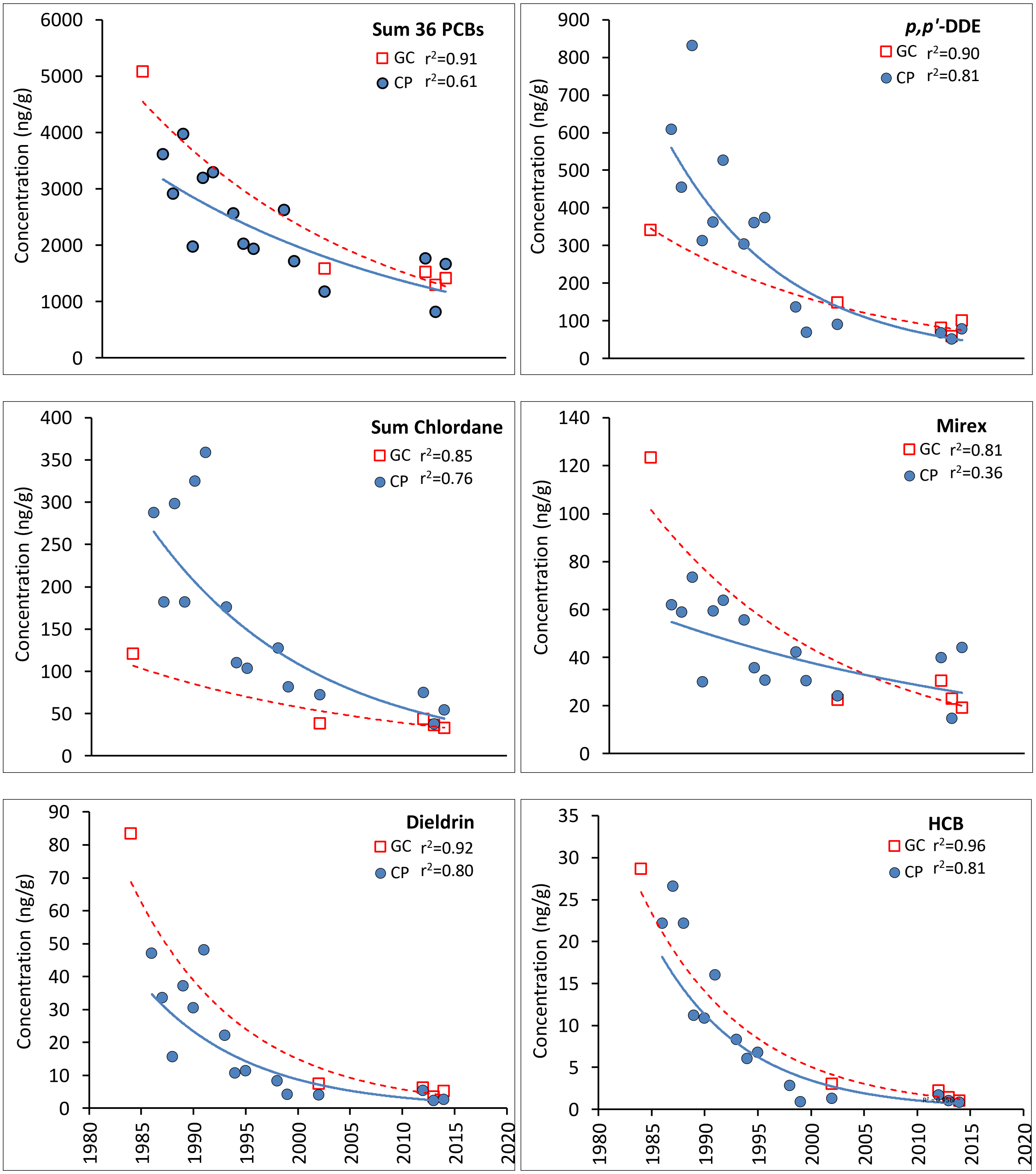
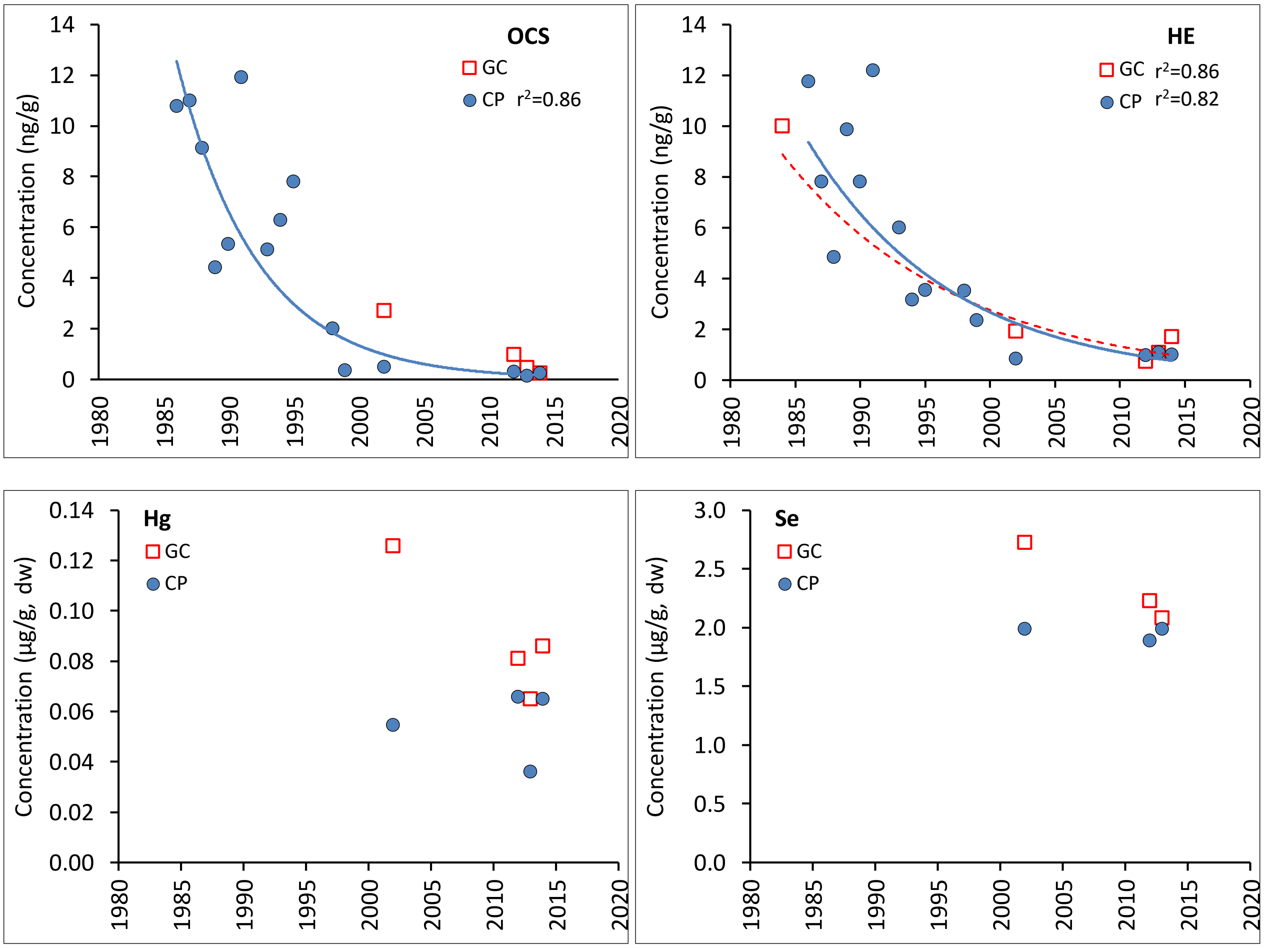


Figure 7 (continued).



Risk to Wildlife As Consumers of Turtle Eggs:

To assess the potential risk for adverse effects on wildlife as consumers of turtle eggs, contaminant burdens in eggs from AOC locations were compared to Canadian tissue residue guidelines for the protection of wildlife consumers of aquatic biota (CCME 2001). Overall, turtle eggs exceeded tissue residue guidelines for total DDT and PCBs for mammals and birds in at least 86% of clutches (i.e., 43 of 50 clutches) from 2012–2014 (Table 4). Concentrations of PCBs reported in eggs were also likely underestimated since non-*ortho* PCBs (another group of dioxin-like PCBs) were not measured in this study and these also contribute to PCB toxicity. Based on estimated methylmercury concentrations in turtle eggs, no clutches exceeded the CCME guideline for methylmercury (Table 4). At the two reference locations, no clutches of eggs exceeded guidelines for mercury and PCBs for mammals and birds from 2012–2014 (based on 11 clutches at Long Point and 19 clutches at upstream sites). Exceedances of the CCME guideline for total DDT were found in clutches from Long Point (45% or 5 of 11 clutches) and at upstream sites (5% or 1 of 19 clutches).

Table 4. Summary of exceedances of Canadian tissue residue guidelines for wildlife consumers of snapping turtle eggs collected from two Hamilton Harbour AOC locations from 2012–2014 (based on 50 clutches of eggs; CCME 2001). Guidelines for PCBs are shown for mammals and birds as consumers of turtle eggs. Tissue residue guidelines are in wet weight concentrations.

Contaminant	Tissue Residue Guideline	% of Exceedences (Number of Clutches)
Total DDT	14.0 ng/g	98% (49)
PCBs-mammals*	0.79 ng TEQ/kg	98% (49)
PCBs-birds*	2.4 ng TEQ/kg	86% (43)
Methylmercury	33.0 ng/g	0% (0)**

*Based on concentrations of three mono-*ortho* PCBs, PCB #105, #118, and #156.
** Estimated, based on linear relationship between concentrations of methylmercury and total mercury in eggs of Great Lakes turtles (ECCC unpublished).

DISCUSSION

Hatching success and hatchling deformities have been frequently used to assess effects of contaminants in snapping turtles in the Great Lakes (Bishop *et al.* 1991, 1998; de Solla *et al.* 2008). Artificial incubation of freshly-laid eggs under controlled conditions in the laboratory are valuable for assessing the importance of intrinsic factors such as contaminants that may induce early embryonic mortality or result in developmental abnormalities at this critical life stage. This approach has been employed in this study over four years with collections of eggs from multiple sites at two primary Hamilton Harbour AOC locations. Combining all years together, mean hatching success of turtle eggs collected from Grindstone Creek and Cootes Paradise was equal to 90.4% and 89.2%, respectively (based on N=27 and 35 clutches). Mean percentages of deformed hatchlings, on a per clutch basis, were equal to 11.1% at Grindstone Creek and 9.7% at Cootes Paradise. Comparisons of the two endpoints with suitable reference locations, i.e., upstream reference sites in 2012 and Long Point from 2013–2016, are also important for assessing AOC-localized effects. Hatching success was similar between eggs collected from AOC locations and reference locations in three of four years. In 2014, hatching success of Cootes Paradise eggs was significantly lower compared to Long Point eggs; this trend however was not found when egg collections targeting the Princess Point area were repeated in 2016. Furthermore, frequencies of deformed hatchlings were also not elevated in egg collections from AOC locations compared to reference locations. Hatching success was significantly lower in eggs collected from Grindstone Creek (mean=59.4%) compared to reference sites in a similar study from 2002–2004, a finding that may have been influenced by the relatively few (5) number of clutches collected (de Solla *et al.* 2008). This reduction in hatching success was not found in clutches from Grindstone Creek in this study where a relatively higher number of clutches were collected. Hatching success of Cootes Paradise eggs in the 2002–2004 study was similar to that reported in this study (mean=85.8%, N=21 clutches; de Solla *et al.* 2008). Percentages of deformed hatchlings in the earlier study with means of 10.7% at Cootes Paradise and 4.0% at Grindstone Creek were either similar or lower than those reported in this study (de Solla *et al.* 2008).

Organochlorine burdens, dominated by sum PCBs, were consistently higher in eggs from the two AOC locations compared to reference locations from 2012–2014 (Table 1). These results are similar to those of de Solla *et al.* (2007) who also reported significantly higher concentrations of these compounds in eggs from these AOC locations compared to inland reference sites in 2001–2004. Sum PCB concentrations in turtle eggs from these AOC locations were also among the highest found relative to other Great Lakes sites in the mid-1980s/early 1990s (Struger *et al.* 1993; Bishop *et al.* 1996). Similar spatial patterns for PCBs were found in surface sediment in the main basin of the Harbour and several fish species from the Harbour compared to other non-AOC areas in Lake Ontario (Labencki 2008; Neff *et al.* 2016). Herring gull embryos that were collected as fresh eggs from a colony in Hamilton Harbour and artificially incubated in the laboratory had significantly higher concentrations of sum PCBs and *p*,*p*'-DDE compared to embryos from colonies on Lake Huron and Lake Ontario in 2013 and 2015 (Hughes *et al.* 2018). As a Great Lakes AOC, this pattern of elevated PCB concentrations (particularly in the certain areas of the Harbour) in sediment, fish and turtle eggs has been well-documented.

Several factors relating to anthropogenic activities and physical attributes of the Harbour have contributed to relatively higher levels of contamination in Hamilton Harbour compared to other Lake Ontario areas. The Harbour is a shallow confined embayment that is surrounded by several major industries and large urban centres with effluent from three (formerly four) wastewater treatment plants that discharge to the Harbour. Three main tributaries drain the entire watershed (approximately 500 km^2 in size) and flow either into Cootes Paradise or directly into the Harbour. Cootes Paradise is connected to the west end of the Harbour by a narrow canal which limits water exchange between the two water bodies. At the east end, the Harbour is connected to Lake Ontario by a narrow shipping channel that also limits the extent of water circulation with the rest of Lake Ontario. In addition to pollutant loadings from industries and wastewater treatment plants, urban and rural runoff, atmospheric input, combined sewer overflows, spills and leachate from landfills have also contributed to increased loadings into the Harbour (Hamilton Harbour Remedial Action Plan 1992). While legislation and abatement programs have significantly reduced inputs from these sources, loadings from historically contaminated sediment continue to provide ongoing sources that are available to biota. There are also localized areas of sediment contamination within the Harbour at Randle Reef, Windermere Basin and Windermere Arm (Harlow and Hodson 1988; Hamilton Harbour Remedial Action Plan 2002). A suspected ongoing source of PCBs was also identified at the Strathearne Avenue boat slip (that discharges to Windermere Arm) and a track-down source investigation was initiated (Labencki 2011). These factors have contributed to exceedances of federal and provincial PCB sediment quality guidelines in sediment in many areas of the Harbour in 2014 and PCB consumption advisory benchmarks for fish in the Harbour from 2005–2013 (Neff *et al.* 2016; Milani *et al.* 2017). For snapping turtles in the Hamilton Harbour AOC, these factors have also contributed to the highest PCB burdens reported in eggs and hatchlings compared to other sites in the Great Lakes basin from 2001–2004 as well as other AOCs in more recent studies on the St. Clair River (2011) and Detroit River (2014–2016; Figure 5).

Contaminant burdens in snapping turtle eggs reflect maternal burdens that are acquired throughout the home range of females prior the egg laying period (Pagano *et al.* 1999). In Ontario, the snapping turtle's home range can vary from a few hectares to a few dozen hectares (Pettit *et al.* 1995; Paterson *et al.* 2012). Home range size may be influenced by food availability which is not likely limited in the AOC (with a large wetland component) based on clutch size estimates. Mean clutch size at AOC locations (40 eggs per clutch) was within the range of typical clutch sizes consisting of 25–45 eggs for snapping turtles (Ernst and Lovich 2009). Home range areas where turtles forage may also differ from nesting areas where females lay eggs and that are better suited to incubation. A tracking study of 15 female turtles in Cootes Paradise in 1990 found that the maximum distance travelled between the residence and the nesting area was 2.0 km (mean=1.05 km; Pettit *et al.* 1995). In addition to differences in foraging activities, individual food preferences of turtles will also influence contaminant burdens in eggs (Bishop *et al.* 1994). Snapping turtles are generalist omnivores and their diet consists of fish, aquatic plants, frogs, insects, juvenile birds, mollusks, and other food items (Lagler 1943). As such, exposure to contaminants such as PCBs will be influenced by the trophic level of prey that turtles are feeding on (e.g., more plant-based diet and insects versus forage fish, larger fish or birds) as well as consumption of prey including large migratory fish that have acquired burdens elsewhere including outside of the AOC.

Organochlorine burdens in snapping turtle eggs likely reflect relatively recent dietary exposure versus long-term exposure to these compounds. Concentrations of PCBs in body tissues of snapping turtles have been shown to increase with increasing size and age however no such pattern of increasing burdens with age is evident in eggs (Hebert *et al.* 1993; Bishop *et al.* 1994). Furthermore, Bishop *et al.* (1994) contend that since clutch mass accounts for a small percentage (7–13%) of female body mass and the period of egg production in snapping turtles occurs over several months, it is probable that lipids required for egg production (and hence organochlorine burdens) are derived from dietary intake rather than utilization of fat stores. Similar rates of decline for both sum PCBs and *p,p'*-DDE between Hamilton Harbour herring gull eggs and turtle eggs from 1986–2012 also suggests that turtles are not accumulating these compounds indefinitely (Hughes *et al.* 2016). In this study, PCB concentrations in eggs were largely similar among eight collection sites in the two AOC locations. This finding is not surprising given the large area of foraging habitat available and locations of egg collection sites that are in relatively close proximity to one another. Collections of turtles from relatively more contaminated areas of the Harbour might reveal relatively higher burdens in foraging turtles from these sites. In 2017, following recent concerns of an ongoing source of PCBs at the Strathearne Avenue boat slip, an attempt was made to trap adult snapping turtles in Windemere Basin and elsewhere in the Harbour for blood sampling to assess PCB exposure. This attempt was not successful as no adults were collected in traps due in part to high water levels in the Harbour that year and general lack of habitat. Further plans for trapping turtles were not initiated as additional sampling sites nearby revealed little prospect for finding turtles.

Despite relatively higher concentrations of PCBs in turtle eggs in the AOC overall, current PCB concentrations in eggs, on average, are well below those reported historically. Large declines in concentrations of PCBs and other organochlorines in turtle eggs since the mid-1980s indicate that exposure to these compounds has decreased in turtles foraging at the two primary AOC locations (Figure 7). Similar large and dramatic temporal declines in these legacy compounds were found in herring gull eggs collected from a nesting colony in the Harbour from 1981–2016 (Hughes *et al.* 2018). Large decreases in concentrations of organochlorines were also found in eggs of double-crested cormorants (*Phalacrocorax auritus*) collected from a colony in the Harbour between 1989 and 2016 (Hughes *et al.* 2018). These results are consistent with long-term temporal patterns for PCBs and other compounds reported in several fish species and suspended sediment in the Harbour (Burniston *et al.* 2016; Neff *et al.* 2016). Such large declines in the bioavailability of these contaminants can be attributed to production bans and restrictions on the use of chemicals, improved industrial practices, and the effectiveness of remedial activities in reducing chemical inputs in Hamilton Harbour.

Consistent with its action as an endocrine-disrupting compound, PCB exposure in turtles has been associated with sex reversal, intersex condition and impacts on embryonic and hatchling development and growth rates (Adams *et al.* 2016). Impacts on any of these traits can also result in subsequent effects on biochemical responses that can impact behaviour and long-term survival of individuals in the population. In contrast to birds, no threshold criteria have been developed for adverse effects on survival and reproduction in reptiles exposed to PCBs. Using thresholds identified for birds as a surrogate threshold for turtles in this study, based on a broad literature review of PCB effects in birds, Hoffman *et al.* (1996) concluded that sum PCB concentrations in the range of 8,000 to 25,000 ng/g in eggs were associated with decreased hatching success for terns and cormorants. Sum PCB concentrations in turtle eggs from AOC collection sites were less than 3,000 ng/g in 92% of clutches, i.e., 45 of 49 clutches, and were well below this lower threshold. Although also below this lower threshold, it is difficult to speculate on potential effects in the four remaining clutches with PCB burdens ranging from 3,000–6,000 ng/g. Subtle effects relating to impacts on health of hatchlings or adults cannot be ruled out in these clutches. It is not clear what factors may have contributed to the unexpected findings reported for the two endpoints at the reference locations (upstream reference sites-2012 and 2013 and Long Point-2016) where total burdens of legacy compounds in eggs were low.

Mercury concentrations in AOC eggs were less than or similar to concentrations in Long Point eggs and overall were among the lowest found relative to concentrations in eggs from other Great Lakes sites (Table 2; Figure 6). Compared to PCBs, relatively lower mercury burdens in turtles in the AOC are consistent with recent trends in fish collected in the Harbour that did not exceed mercury consumption advisory benchmarks (Neff *et al.* 2016). Similar to PCBs, few studies have examined thresholds associated with the toxic effects of mercury in reptiles. Hopkins *et al.* (2013) demonstrated that total mercury in snapping turtle eggs was negatively correlated with hatching success through increased egg infertility and embryonic mortality. They found that an average mercury concentration of 3.0 µg/g dw in eggs from a mercury-contaminated study site in Virginia was associated with a 12% reduction in hatching success compared to the reference sites. Mercury concentrations in AOC eggs were at least an order of magnitude lower than this concentration with a maximum dry weight concentration of 0.15 µg/g. Hopkins *et al.* (2013) used, as a surrogate threshold-effect level for turtles, the predicted mercury threshold of 0.6 µg/g ww in bird eggs as being protective against adverse reproductive effects for 95% of non-marine avian species (Shore *et al.* 2011). The maximum mercury concentration in AOC eggs in this study (0.028 µg/g ww) was well below this threshold concentration. Mercury concentrations were not notably elevated in turtle eggs from AOC locations and were likely not sufficiently elevated to adversely influence reproduction or development in snapping turtles in the AOC.

As essential metals, selenium, copper, and zinc are necessary for various biochemical and physiological functions in living organisms. These metals were detected in 80-100% of turtle egg samples from study locations with the exception of selenium in eggs from the two reference locations which were detected less frequently in 2013 (Appendix A2). Selenium was the only metal with significantly higher concentrations in eggs from AOC locations compared to reference locations in the two study years. Ware *et al.* (2011) reported that selenium concentrations increased over the winter in liver of greater scaup (*Aythya marila*) collected in Hamilton Harbour and suggested that elevated selenium concentrations could be related to the presence of coal-burning steel mills. This may be true also for snapping turtles. However, selenium concentrations in turtle eggs from AOC locations were well below the threshold of 3 µg/g ww in bird eggs that is associated with reproductive problems (Heinz 1996). At sufficiently high concentrations, non-essential metals such as lead, cadmium, and arsenic are associated with adverse health effects in reptiles (Grillitsch and Schiesari 2010). These three metals were detected infrequently, i.e., in 0–30% of egg samples from AOC locations (Appendix A2). Similar to mercury, concentrations of other metals in eggs were likely not sufficiently elevated to influence reproduction or development in turtles in the AOC.

Evidence from this study suggests that wildlife as consumers of snapping turtle eggs may be at an increased health risk due to elevated concentrations of total DDT and PCBs expressed as TEQs (Table 4). In addition, PCB concentrations in eggs were also underestimated since non-*ortho* PCBs (another group of dioxin-like PCBs) were not measured in this study and these also contribute to TEQ PCB toxicity. There appears to be no health risk to consumers of eggs for methylmercury based on estimated concentrations.

In conclusion, the results of this four-year study suggest there was no evidence of impairment for the two reproduction and development endpoints measured in snapping turtles in the Hamilton Harbour AOC that could be attributable to local contamination within the AOC. Hatching success was similar among eggs collected from AOC locations and reference locations in three of four years. One year of reduced hatching success in Cootes Paradise eggs, largely attributable to a single clutch from Princess Point, was not repeated in subsequent collections and clutch failure in this one clutch was not associated with elevated contaminant burdens. Frequencies of deformed hatchlings were also not elevated in egg collections from AOC locations compared to reference locations. Eggs from the two AOC locations had the highest organochlorine burdens, dominated largely by PCBs, compared to those from reference locations and other Great Lakes AOCs. Large declines in concentrations of sum PCBs and most other organochlorines in eggs since the mid-1980s indicate that exposure to these compounds has decreased substantially. Mercury concentrations were not notably elevated in eggs from AOC locations compared to reference locations. Concentrations of PCBs and mercury in turtle eggs were not sufficiently elevated to adversely impact the reproductive success and development of snapping turtles in the Hamilton Harbour AOC. Nevertheless, current concentrations of PCBs and total DDT in eggs exceeded tissue residue guidelines and are of concern to wildlife as consumers of turtle eggs in the AOC.

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Appendix A1. Mean concentrations of organochlorines and sum PBDEs (SD, ng/g, lipid weight) in eggs of snapping turtles from Grindstone Creek and Cootes Paradise in the Hamilton Harbour AOC and two references locations at Long Point on Lake Erie and sites upstream of the AOC in 2012, 2013, and 2014. Mean concentrations (SD) in newly hatched turtles in clutches from Princess Point (Cootes Paradise) in 2016 are indicated by a "*". Sum PCBs are based on the sum concentrations of 35–62 congeners depending on the year of analysis. Sum PBDEs are based on the sum concentrations of 15 or 25 individual or coeluting BDE congeners. N represents the number of clutches of eggs analyzed with the exception of eggs from Grindstone Creek and Long Point in 2014 that were analyzed as a single composite pool.

Location	AOC /REF	N	Year	% Lipid	Sum PCBs	*p,p'*-DDE	Sum Chlordane	Dieldrin	OCS	HCB	HE	Mirex	Sum PBDEs
Grindstone Creek	AOC	9	2012	5.58 (1.31)	30169 (20005)	1426 (739)	819 (694)	115 (47.4)	17.2 (14.0)	41.9 (30.4)	13.7 (9.2)	531 (565)	829 (392)
Grindstone Creek	AOC	10	2013	5.25 (0.51)	25934 (14952)	1054 (782)	651 (536)	65.7 (37.6)	8.0 (7.8)	25.6 (16.0)	19.6 (13.9)	406 (441)	809 (536)
Grindstone Creek	AOC	1	2014	8.06	19007	1232	410	63.5	2.9	12.3	21.1	236	818
Cootes Paradise	AOC	10	2012	5.64 (1.24)	37460 (25659)	1274 (621)	1418 (1023)	99.4 (53.2)	5.0 (3.6)	30.1 (14.9)	18.9 (15.6)	748 (544)	989 (616)
Cootes Paradise	AOC	10	2013	5.79 (1.16)	16592 (9166)	944 (613)	708 (442)	43.5 (28.4)	2.5 (2.8)	19.6 (12.8)	20.2 (13.8)	271 (190)	812 (629)
Cootes Paradise	AOC	10	2014	5.36 (1.26)	33243 (23932)	1440 (726)	932 (970)	46.6 (28.9)	4.2 (2.7)	15.5 (7.7)	17.0 (15.1)	790 (743)	1149 (1169)
Cootes Paradise	AOC	6*	2016	2.48 (0.40)	30316 (8756)	1322 (559)	682 (195)	71.9 (15.7)	3.1 (1.2)	48.0 (8.3)	11.2 (4.5)	263 (232)	1152 (281)
Long Point	REF	10	2013	5.36 (0.59)	535 (510)	311 (195)	61.7 (85.0)	20.0 (14.9)	ND	4.6 (2.0)	1.9 (2.1)	3.2 (2.7)	19.0 (15.1)
Long Point	REF	1	2014	8.34	1267	922	156	78.1	ND	2.7	22.3	ND	77.5
Upstream Sites	REF	10	2012	5.91 (1.81)	209 (189)	75.9 (57.5)	18.8 (13.8)	18.4 (11.9)	ND	5.2 (3.5)	ND	4.6 (2.8)	13.9 (16.8)
Upstream Sites	REF	10	2013	5.58 (1.45)	201 (108)	70.5 (74.6)	71.5 (131.7)	34.5 (10.5)	ND	4.4 (1.7)	1.2 (0.7)	ND	25.3 (34.9)

Appendix A2. Mean concentrations of metals (SD, µg/g, dry weight) in snapping turtle eggs from Grindstone Creek and Cootes Paradise in the Hamilton Harbour AOC and two references locations at Long Point on Lake Erie and sites upstream of the AOC in 2012 and 2013, where data are available. The percentage of egg samples with a concentration above the respective practical detection limit (PDL) is also shown. Blue cells indicate that no egg samples had concentrations above the PDL and concentrations reported between the theoretical detection limit (TDL) and PDL were used to determine a mean concentration in these samples. N represents the number of clutches of eggs analyzed. ND indicates that concentrations were below the TDL in all egg samples.

Location	Year	N		Ba	Cu	Fe	Ga	Li	Mn	Ni	Rb	Se	Sr	V	Zn	As	Co	Mo	Pb
Grindstone Creek	2012	9	**Mean**		1.8				1.5	0.24	2.9	2.2			74.8	0.07	0.02		0.01
			SD		0.3				0.4	0.05	1.4	0.6			4.8	0.03	0.01		0.01
			%>PDL		100%				100%	100%	100%	100%			100%	0%	0%		0%
	2013	10	**Mean**	15.9	2.0	74.3	0.56	0.06	1.9	0.29	3.0	2.1	18.4	0.18	81.5	0.17	0.01	0.08	0.01
			SD	6.5	0.9	9.4	0.23	0.04	0.4	0.06	1.3	0.5	9.7	0.03	7.7	0.07	0.02	0.04	0.04
			%>PDL	100%	90%	100%	100%	80%	100%	100%	100%	80%	100%	100%	100%	30%	0%	0%	0%
Cootes Paradise	2012	10	**Mean**		2.0				1.3	0.24	3.4	1.9			79.7	0.11	0.01		0.01
			SD		0.5				0.7	0.04	1.1	0.3			9.1	0.06	0.01		0.01
			% >PDL		100%				100%	100%	100%	100%			100%	20%	0%		0%
	2013	10	**Mean**	20.4	2.7	77.6	0.72	0.12	1.3	0.34	3.6	2.0	57.2	0.16	73.6	0.17	**ND**	0.06	0.01
			SD	10.8	0.5	9.9	0.38	0.03	0.4	0.12	1.5	0.5	35.7	0.01	7.1	0.09		0.03	0.04
			%>PDL	100%	100%	100%	100%	100%	100%	100%	100%	80%	100%	100%	100%	30%		0%	0%
Long Point	2013	10	**Mean**	24.0	2.1	67.1	0.86	0.09	1.5	0.36	4.3	1.3	24.2	0.20	69.5	0.11	**ND**	0.05	0.01
			SD	8.6	0.8	9.6	0.30	0.02	0.4	0.09	2.3	0.4	7.7	0.03	15.2	0.05		0.04	0.02
			%>PDL	100%	90%	100%	100%	100%	100%	100%	100%	20%	100%	100%	100%	0%		0%	0%
Upstream Sites	2012	9	**Mean**		1.8				1.6	0.24	2.7	1.3			76.9	0.07	0.03		0.03
			SD		0.6				0.5	0.02	0.8	0.2			7.1	0.04	0.02		0.02
			%>PDL		100%				100%	100%	100%	100%			100%	11%	0%		0%
	2013	10	**Mean**	24.4	1.4	69.0	0.86	0.10	1.9	0.38	2.9	1.4	30.1	0.16	83.0	0.10	**ND**	0.07	0.18
			SD	8.2	0.4	6.8	0.28	0.04	0.7	0.10	0.9	0.4	10.3	0.03	7.6	0.04		0.04	0.37
			%>PDL	100%	80%	100%	100%	100%	100%	100%	100%	50%	100%	70%	100%	0%		0%	20%