ST. CLAIR RIVER AREA OF CONCERN (ONTARIO)

Status Recommendation Report for BU #5: Bird or Animal Deformities or Reproduction Problems

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St. Clair River
Remedial Action Plan

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Executive Summary

Several studies were conducted by Environment and Climate Change Canada, in collaboration with Walpole Island First Nation, to determine if there is evidence of impairment of wildlife reproduction and development resulting from elevated contaminant exposure in the St Clair River Area of Concern (Ontario). This work is directly relevant to assessment of Beneficial Use (BU) #5 - *Bird or Animal Deformities or Reproduction Problems* – which currently is designated as "Requires Further Assessment". Two aquatic sentinel species were selected for assessment purposes, the northern leopard frog (*Rana pipiens*) and the common snapping turtle (*Chelydra serpentina*), both of which are known to be sensitive to the effects of contaminants. Hatching success and deformity rates were selected as biological endpoints for assessment. The results of these studies conducted between 2006 to 2014 have been detailed extensively in two earlier reports (Hughes *et al.* 2014, 2015). The purpose of this summary report is to provide a brief overview of findings that pertain specifically to the assessment of BU #5 which will provide the basis for an official status change for this BU in the St. Clair River AOC (Ontario).

Three sets of criteria were used for assessing reproduction and deformities in wildlife: i) hatching success and deformity rates in frog embryos and turtle hatchlings; ii) deformity rates in wild populations of newly-transformed young-of-year frogs, and; iii) contaminant concentrations in whole bodies of frogs and turtle eggs that were used to support the assessment results for the two biological endpoints. Exposure studies were conducted on fertilized frog eggs exposed to water (2007) and water and sediment (2011) from several AOC locations to assess the impact of exposure of these media on egg hatchability and amphibian development. The results of these studies indicated that mean hatching success of leopard frog eggs was high (>98%), mean percentages of embryos with a deformity were low to moderate (<7%) and, importantly, rates of these two endpoints were statistically similar to rates reported at non-AOC reference sites. Based on 16 of 18 surveys of wild populations of froglets conducted in 2006, 2007, 2011 and 2014, deformity rates (represented as a percentage) were low overall at AOC locations and below the 5% threshold considered elevated in wild populations of amphibians. In two surveys conducted in 2007 where the threshold was exceeded, no evidence of elevated deformity rates was found at these two locations in 2011 and/or 2014. Therefore, exceedences reported above 5% at these two sites in 2007 were not consistently found in later years.

Forty-one clutches of snapping turtle eggs were collected from many locations throughout Walpole Island delta in 2011 and were artificially incubated in the laboratory and assessed for hatching success of eggs and deformity rates of hatchlings. Consistent with the results found for leopard frogs, mean hatching success of snapping turtle eggs was high (93.5%), the mean percentage of deformed hatchlings was low (7.7%) and no significant differences were found for either of these endpoints compared to rates found at the non-AOC reference site.

Organochlorine burdens in frogs and turtle eggs for four compounds of concern in the AOC, i.e., PCBs, dieldrin, HCB and OCS, were generally ranked as intermediate relative to other Great Lakes AOCs and references sites. Sum PCBs and dieldrin in wild frogs from the AOC were well below those associated with toxicity. Concentrations of mercury, as another contaminant of concern, varied among St. Clair River AOC locations in frogs and turtle eggs and potential effects associated with the highest levels of

exposure are not known. Mercury concentrations in frogs and turtles were not notably elevated relative to other sites with a history of local point sources of mercury pollution. Based on the criteria used to assess wildlife reproduction and deformities in these multi-year studies, there was no evidence of impairment for these biological endpoints in two aquatic wildlife sentinel species in the St. Clair River AOC (Ontario).

The results of these studies suggest there is sufficient evidence to support a recommendation to designate the status of BU #5 - *Bird or Animal Deformities or Reproduction* – as "Not Impaired" in the St. Clair River Area of Concern (Ontario).

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1. Introduction

In 1857 the discovery of crude oil within the Sarnia area led to the establishment of several refineries. In 1942, Polymer Corporation was established and manufactured synthetic rubber. The combined success of oil refining and chemical production intensified and with access to raw resources and transportation, Sarnia became the largest petro-chemical complex in the province of Ontario and one of three of Canada's oil refining centres. In fact, the 1971 image of Sarnia's "chemical valley" was printed on the Canadian ten dollar note to illustrate its economic importance in Canada.

In the 1980's, scientific research and monitoring was conducted to identify sources of pollution and the extent of the environmental degradation to the St. Clair River. It was determined that the success of Sarnia's industrial complex and municipal growth resulted in severe environmental degradation of the aquatic environment. Industrial effluent discharged to the St. Clair River contained organic contaminants such as hexachlorobenzene (HCB), octachlorostyrene (OCS), polychlorinated biphenyls (PCBs) and toxic metals such as mercury. Inadequate municipal infrastructure resulted in high bacteria levels and unsightliness of the river and the use of dieldrin as a pesticide in agriculture and washed into tributaries further added to the degradation of the local aquatic environment. In 1987, the St. Clair River was designated as an "Area of Concern" (AOC) as it "*failed to meet the general or specific objectives of the Great Lakes Water Quality Agreement (GLWQA) where such failure has caused, or is likely to cause, impairment of beneficial uses or the area's ability to support aquatic life."* Of the 14 "beneficial uses" assessed under the GLWQA, nine were deemed "impaired" in the St. Clair River AOC including Beneficial Use (BU) #5 - *Bird or Animal Deformities or Reproduction Problems.* As the name of this beneficial use implies, it is intended to evaluate whether contaminants generated from within an AOC were adversely impacting reproduction (e.g. hatching success) or development (physical deformities) in local birds or animals.

In accordance with the provisions of the GLWQA when an area is identified as an AOC, a remedial action plan (RAP) is developed and then implemented in stages: the first stage identifies pollution sources and extent of environmental impacts; the second stage details the creation and implementation of restoration actions for those BUs determined to be impaired, and; the final stage is when all impairments are restored. The *1991 Stage 1 Report* summarized the conditions of water, sediment and biota (which included benthic invertebrates, fish and wildlife) and concluded that mercury, HCB, PCBs, dieldrin and OCS were accumulating in wildlife tissue (Ontario Ministry of the Environment and Michigan Department of Environmental Quality 1991). These five compounds were identified as contaminants of concern as they are known to *bio-accumulate* and *bio-magnify* through the food web. These are of particular concern for aquatic-feeding wildlife as increased contaminant exposure is frequently associated with reproduction and development problems (Bishop *et al.* 1998). Although there was no evidence or reports of reproductive problems or physical deformities reported or observed within the St. Clair River AOC, an "abnormally high" rate of mouthpart deformities among chironomids, a benthic organism, was found in areas below the Sarnia industrial complex. This finding resulted in the "Impaired" designation for BU #5 - *Bird or Animal Deformities or Reproduction Problems* (Ontario Ministry of the Environment and Michigan Department of Environmental Quality 1991). The 1995 *Stage 2 – Recommended Plan* identified six specific qualitative goals, one of which pertained to ecosystem

health and the need to "*attain and maintain healthy, diverse and self-sustaining biological communities and habitats and ensure that there are no negative impacts on health of local populations due to water quality."* (Ontario Ministry of Environment and Energy and Michigan Department of Natural Resources 1995). AOC-specific water, sediment and biota quality guidelines, referred to as "yardsticks", were established for various compounds originating from within the AOC. Nine biota yardsticks were specifically developed using the most current guidelines and other resources available and tended to reflect guidelines associated with the protection of higher trophic level consumers and not thresholds associated with adverse reproduction or growth effects in wildlife. While development of these biota yardsticks was instrumental in identifying priority contaminants of concern in wildlife due to bioaccumulation and biomagnification potential, they also helped to focus RAP restoration efforts and create delisting criteria for impaired beneficial uses including BU #5. The 1995 *Stage 2 – Recommended Plan* indicated that BU #5 would no longer be impaired "*when chironomid mouthpart anomalies occur at rates similar to incidences in "control" populations* (Ontario Ministry of Environment and Energy and Michigan Department of Natural Resources 1995). This restoration target was consistent with the BU delisting guidance provided by the International Joint Commission (IJC) which suggested a comparison approach using sentinel species for this particular beneficial use.

The S*t. Clair River Remedial Action Plan – Stage 1 1997 Update* report documented improvements within the AOC and highlighted downward trends in industrial loadings, environmental contaminant concentrations and frequency of chemical spills (Geomatics International Inc. 1998). Wildlife studies conducted between 1991 and 1995 revealed that PCBs, dieldrin, and OCS concentrations in some species exceeded respective biota yardstick concentrations (Geomatics International Inc. 1998). The yardstick for mercury was also exceeded in some fish species as well causing concern for fish-eating wildlife. Despite these yardstick exceedances, Environment and Climate Change Canada (ECCC) biologists commented that concentrations were generally below respective thresholds where reproductive success would be adversely affected based on available data, observations and studies done elsewhere across the Great Lakes (Geomatics International Inc. 1998). Furthermore, the *Stage 1 1997 Update* report recommended that the issue of chironomid mouthpart deformities be evaluated as part of the assessment for the *Degradation of Benthos* BU since it is a better indicator of sediment quality and benthic community than reproductive health in wildlife. This report concluded with the recommendation to re-designate BU#5 to "Not Impaired". However, the recommendations did not proceed in part due to a formal binational process established in 1998 for (re)designating BUIs. This process required supporting documentation and data to validate the change in status. It also required consultation with the RAP partners and the newly established Four Agency Management Committee. To fulfill the expectations of the new process, additional studies to assess the *Bird or Animal Deformities or Reproduction Problems* BU were necessary.

In 1999, snapping turtle (*Chelydra serpentina*) eggs were collected from Walpole Island to examine reproductive success and measure contaminant burdens. Upon artificial incubation of these eggs in the laboratory, there was no evidence of reproductive impairment since hatching success and rates of hatchling deformities were not significantly different between clutches of eggs from the AOC and the reference site at Algonquin Provincial Park (Ashpole 2003). However, PCB concentrations in snapping

turtle eggs collected from within the AOC in 1999 as well as from the un-impacted pristine site in Algonquin Provincial Park over several years exceeded the biota yardstick guideline of 2.5 ng/g (Ashpole *et al.* 2004). This suggested that the biota yardstick for PCBs was too conservative and impractical. In further support of this, the PCB guideline developed by the New York State Department of Environmental Conservation (NYSDEC) to protect fish-eating wildlife was an order of magnitude higher than the biota yardstick (110 ng/g; Newell *et al.* 1987). Similarly, NYSDEC guidelines developed for dieldrin and HCB were at least three times higher than the respective biota yardsticks (Newell *et al.* 1987). Studies conducted on wild mink (*Mustela vison*) trapped in the St. Clair River AOC in 2002 found that concentrations of sum PCBs and mercury in liver did not exceed thresholds associated with effects on reproduction and/or survival (Restum *et al.* 1998; Wolfe *et al.* 1998; Martin *et al.* 2006, 2011). Although these studies suggested overall that contaminant burdens were not adversely affecting reproduction in local aquatic animals (turtles and mink), the *St. Clair River RAP Progress Report Volume 1 – Synthesis Report* concluded that there was insufficient evidence to fully characterize this BU (Mayne 2005).

Environment and Climate Change Canada, in collaboration with Walpole Island First Nation, conducted wildlife research studies between 2006 and 2014 to examine whether there was evidence of development or reproductive challenges in birds or animals within the AOC. The results of these comprehensive studies provide the basis for the Canadian Remedial Action Plan Implementation Committee (CRIC) to support a recommendation to formally provide a designation for the current status of BU #5 in the St. Clair River AOC (Ontario). Although previous studies compared contaminant concentrations in biota to corresponding biota yardsticks, these yardsticks do not reflect thresholds associated with adverse effects on reproduction or growth in wildlife; consequently, this assessment will not use biota yardsticks for assessment of this beneficial use but rather are based on the results of two comprehensive wildlife studies.

2. Assessment Approach

To determine the status of BU #5 - *Bird or Animal Deformities or Reproduction Problems* - an assessment approach consistent with the guidance provided by the IJC was developed whereby sentinel wildlife species would be used to assess deformities and reproduction problems (IJC 1991). Sentinel species selected for the St. Clair River AOC are the northern leopard frog (*Rana pipiens*) and the common snapping turtle.

Northern leopard frogs have a biphasic life cycle with the first part of their life in water as an egg and tadpole after which, at the time of metamorphosis, they leave the breeding pond as a frog and occupy terrestrial habitat. This species is widespread and native with a semi-permeable skin which makes it sensitive to poor water quality. The common snapping turtle is semi-aquatic top predator with a predominantly piscivorous (fish-eating) diet. It commonly inhabits wetlands on the Great Lakes, has a wide geographic distribution and a relatively small home range. Both species have a close connection to the aquatic environment and, as well, accumulate and are sensitive to the effects of contaminants. Consequently, both species are good bio-indicators of environmental contaminant conditions (Bishop *et al.* 1998; Huang *et al.* 1999; de Solla *et al.* 2007; 2008; Hopkins *et al.* 2013).

Environment and Climate Change Canada (ECCC), in collaboration with Walpole Island First Nation, conducted extensive studies to assess reproductive health and development in northern leopard frogs (2006, 2007, 2011, and 2014) and snapping turtles (2011). Studies of frogs were conducted at two important development stages: 1) early embryonic stage in the laboratory to assess hatching success and embryonic deformities and 2) early transformation stage following metamorphosis to assess deformities in wild populations. Snapping turtle eggs were collected from numerous locations in the AOC and incubated in the laboratory to assess hatching success of eggs and rates of hatchling deformities. Numerous contaminants were measured in these species as well as collections of water and sediment to determine quality and, in effect, assess potential exposure in aquatic wildlife. Detailed information on study methods and parameters measured in these studies are in two reports included in the Supplemental Information for further reference:

1. *Reproductive Health and Development in Northern Leopard Frogs (Rana pipiens) in the St. Clair River Area of Concern (Ontario)* by Hughes *et al.* 2014.
2. *An Assessment of Reproductive Health and Development of Snapping Turtles (Chelydra serpentina) from the Walpole Delta in the St. Clair River Area of Concern* by Hughes *et al*. 2015.

This status re-designation report serves to provide a brief overview of these studies and highlight the findings that pertain to the assessment of BU #5 in the St. Clair River AOC (Ontario). Specifically, three sets of criteria were used for assessing reproduction and deformities in wildlife:

i) *Hatching success and deformity rates* in frog embryos and turtle hatchlings were compared to non-AOC reference sites, in this case, at upstream sites on Lake Huron for frogs and inland from Lake Huron for turtles.

ii) *Deformity rates* of the northern leopard frog were examined to determine whether they exceed the threshold deformity rate of 5% considered elevated in wild amphibian populations.

iii) *Contaminant concentrations* of whole body frogs and turtle eggs (which are representative of maternal body burdens) were compared to contaminant levels found at reference sites, other Great Lakes sites, and scientific studies where toxic effects including adverse effects on development and reproduction have been found.

This report will focus on the five compounds of historic concern in the St. Clair River AOC, i.e., PCBs, dieldrin, HCB, OCS and mercury (Ontario Ministry of Environment and Energy and Michigan Department of Natural Resources 1995). In addition, the organochlorine pesticide DDT (1,1'-(2,2,2-trichloro-1,1-ethanediyl)bis(4-chlorobenzene) has also been reported in wildlife in the AOC and will also be reported on here (Ontario Ministry of the Environment and Michigan Department of Environmental Quality 1991). All of these compounds are toxic, persistent and known to bioaccumulate in biota. Elevated levels of these compounds have been associated with impacts on development and reproduction in amphibians and reptiles (e.g., Cooke 1972; Schuytema *et al.* 1991; Bishop *et al.* 1998; Savage *et al.* 2002; Unrine *et al.* 2004; Hopkins *et al.* 2013). Statistical methods and detailed results and discussion are also provided in the two reports above; statistical significance is determined as $p<0.05$.

3. Northern Leopard Frogs Studies

Two types of studies were conducted using the leopard frog to assess reproduction problems and deformities:

i) Frog embryonic exposure studies: to examine hatching success and deformities in frog embryos under controlled laboratory conditions whereby:
   a. Fertilized northern leopard frog eggs were raised in *water* collected from eight locations from within the St. Clair River AOC (2007).
   b. Fertilized northern leopard frog eggs were raised in *water and sediment* collected from five other AOC locations (2011).

ii) Deformity surveys: 18 surveys of newly-transformed young-of-year frogs (also called "froglets") were conducted in the summer for four years to visually assess froglets for gross deformities (2006, 2007, 2011, and 2014).

Contaminant burdens in whole bodies of frogs were determined in 2006 and 2007 to assess whether current burdens were sufficiently elevated to potentially impact reproduction or development. Non-AOC reference sites were selected for comparison purposes at Port Franks (43°14'03.20"N, 81°53'53.23"W) and Wood Road (43°10'39.24"N, 82°01'09.89"W) and both are situated on the south shore of Lake Huron and upstream of the St. Clair River AOC. Contaminant burdens in frogs from the Detroit River AOC, where similar assessments have been performed, are provided for context.

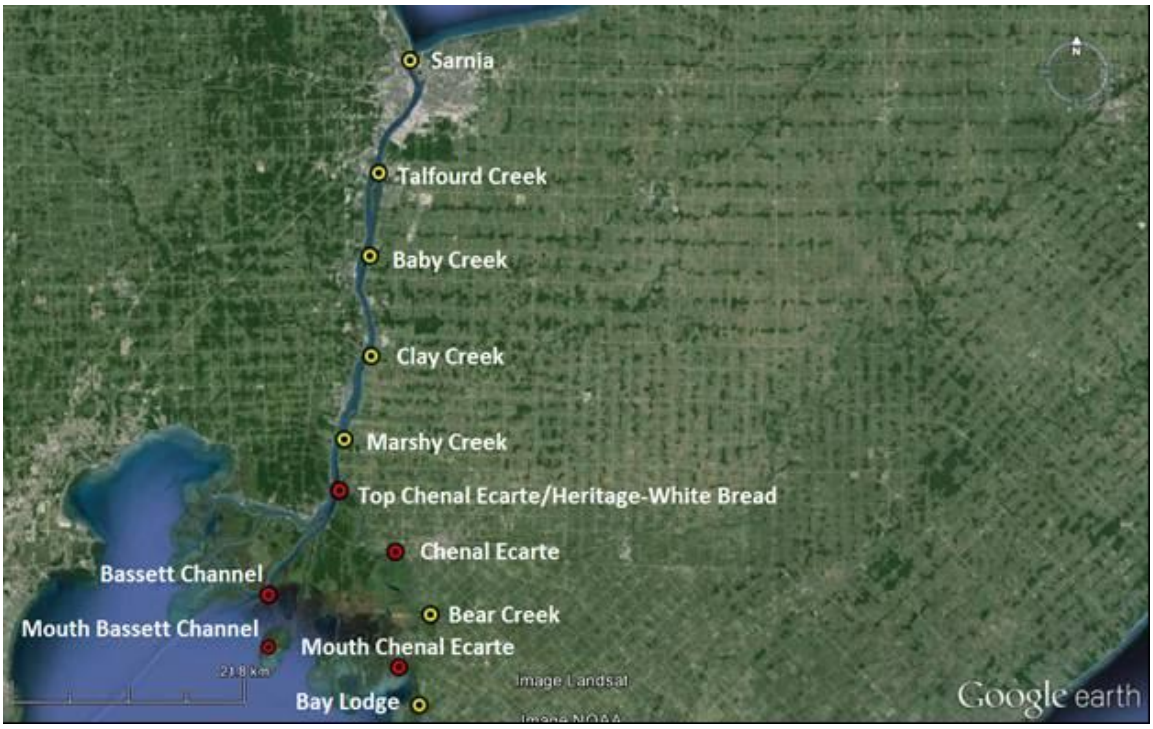
Frog Embryonic Exposure Studies

The minimum requirement for amphibian reproduction in an area is that viable eggs deposited at a site can successfully develop into free swimming tadpoles. To assess the impact of AOC water quality on amphibian development, northern leopard frog eggs were raised in water (2007) collected from eight locations along the St. Clair River from Sarnia to Heritage-White Bread, Bear Creek, and Bay Lodge, as well as the Lake Huron reference site (Wood Road; **Figure 1**). Since contaminated sediment can also be a significant source of contamination to amphibians, another exposure study was conducted in 2011 in which eggs were raised in water and sediment collected from five AOC sites in the Walpole Island delta and the Lake Huron reference site (Port Franks; **Figure 1**). This is an adaptation of the standard FETAX test which is used to assess water quality for both acute toxicity and teratogenicity; therefore, this test is ideal for the assessment of both reproductive impairment and developmental deformities following exposure to contaminants.

Fertilized eggs used in both studies were collected in the spring as fresh egg masses from a reference population, Beverly Swamp in Flamborough Township, Ontario (43°22'0.48" N, 80°6'58.88" W). This reference site has a healthy population of frogs and eggs are readily available. This site is ideal since it has minimal contaminant input from agricultural, industrial, or municipal runoff or effluent sources. Collections of eggs from this site have been used for similar exposure studies done by ECCC for other Great Lakes AOCs.

For both exposure studies, eggs were raised in one liter hexane-cleaned glass jars containing 350 ml of fresh water collected from each of the study sites. Every other day, ten liters of water were collected

Figure 1. Study locations within the St. Clair River AOC where water (yellow circles) and sediment (red circles) samples were collected for the embryonic exposure studies in 2007 and 2011. Note that the Heritage-White Bread water collection location (yellow circle) is hidden behind Top Chenal Ecarte (red circle).



from each of the study sites, placed into chemically clean four liter glass jugs and transported back to the laboratory where they were stored at room temperature. For the water and sediment study, five AOC locations were selected based on a gradient of chemical contamination determined in sediment collected in 2005 (Great Lakes Institute for Environmental Research 2008). Sediment was collected using a mini-ponar and placed in jars and water was collected as described above. Eggs were placed in egg holders to prevent the eggs from coming in direct contact with the sediment. Five replicate jars containing 29-33 eggs per replicate were prepared for each site for each experiment. For each replicate, eggs were checked and the water changed every 24 hours. Exposure studies were conducted at room temperature.

For both exposure studies, eggs were raised through hatching until they developed into free swimming tadpoles, Gosner stage 25, after approximately seven days. Tadpoles were then counted, euthanized using an overdose of MS-222, and preserved in 10% buffered formalin for assessment of deformities. Hatching success were calculated for each replicate as the number of eggs hatched relative to the number of eggs raised and rates of deformed embryos were calculated as the number of deformed embryos relative to the number of eggs hatched.

Hatching success was high for eggs raised in water from eight St. Clair River AOC locations with mean hatching success ranging from 98.0% at Sarnia to 100.0% at Heritage-White Bread and Bear Creek (Figure **2a**). No significant difference in hatching success was found among study sites including the

Figure 2a. Mean hatching success (SD) of northern leopard frog eggs in raised in water from eight St. Clair River AOC locations and one upstream reference site on Lake Huron (Wood Road). Five replicates of 29-32 eggs were tested per site in 2007.

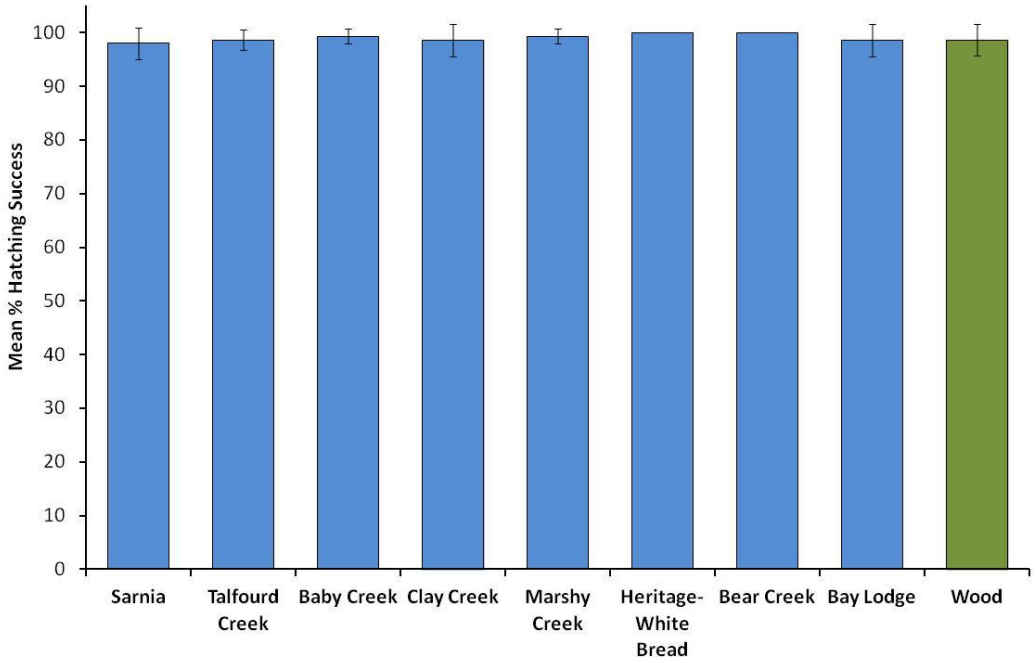
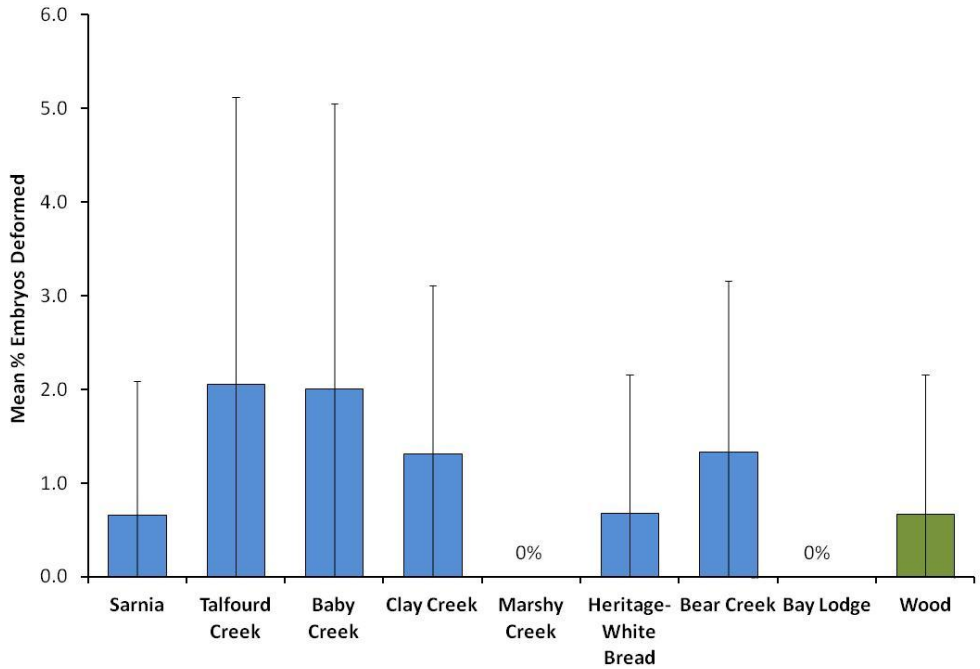


Figure 2b. Mean percentages (SD) of northern leopard frog embryos with a deformity when raised in water from eight St. Clair River AOC locations and one upstream reference site on Lake Huron (Wood Road) in 2007.



Wood Road reference site. In addition, mean percentages of embryos with a deformity were low ranging from 0% at Marshy Creek and Bay Lodge to 2.1% at Talfourd Creek and, similarly, there was no significant difference in the rates of deformed embryos among study sites (**Figure 2b**).

Hatching success was also high in eggs exposed to sediment and water collected from five St. Clair River AOC locations and the Port Franks reference site in 2011 with means ranging from 98.8% to 100.0% (**Figure 3a**). No significant difference in hatching success was found among sites. Mean percentages of embryos with a deformity were low to moderate ranging from 3.8% at the Port Franks reference site to 7.0% at the mouth of Bassett Channel (**Figure 3b**). No significant difference in the rates of deformed embryos was found among study sites.

Deformities in Wild Populations

Surveys of wild frog populations were conducted to assess the presence of gross skeletal deformities in newly-transformed young-of-year frogs (or "froglets"). These surveys were conducted in July when froglets had just completed metamorphosis. Ten AOC locations and two upstream Lake Huron reference were surveyed in 2006, 2007, 2011, and 2014 **(Figure 4)**. Froglets were collected by walking along the shoreline and capturing them with a dip-net or by hand and held in coolers of water until they could be assessed. Deformities were scored visually for the presence of gross morphological deformities and frogs were released at the site where they were caught.

Figure 3a. Mean hatching success (SD) of northern leopard frog eggs raised in water and sediment collected from five St. Clair River AOC locations and one upstream reference site on Lake Huron (Port Franks) in 2011. Five replicates of 30-33 eggs were tested per site.

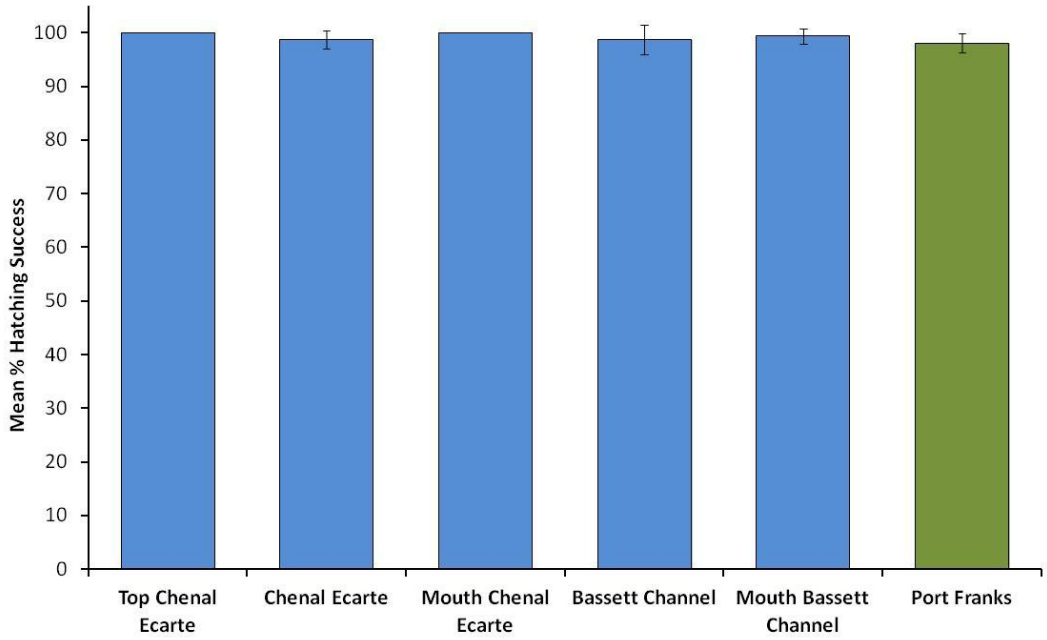


Figure 3b. Mean percentages (SD) of northern leopard frog embryos with a deformity when raised in water and sediment collected from five St. Clair River AOC locations and one upstream reference site on Lake Huron (Port Franks) in 2011.

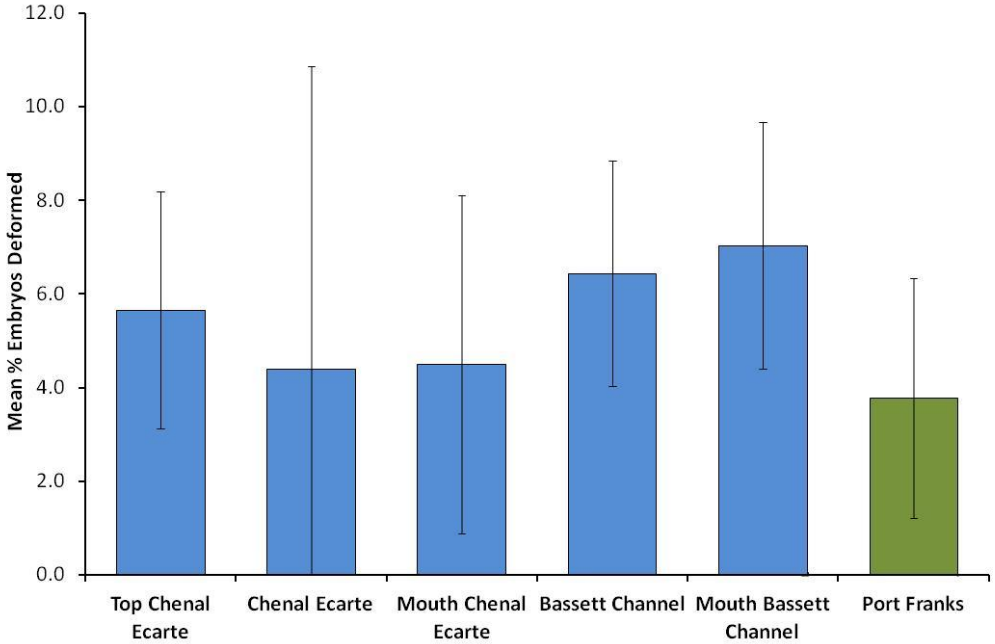
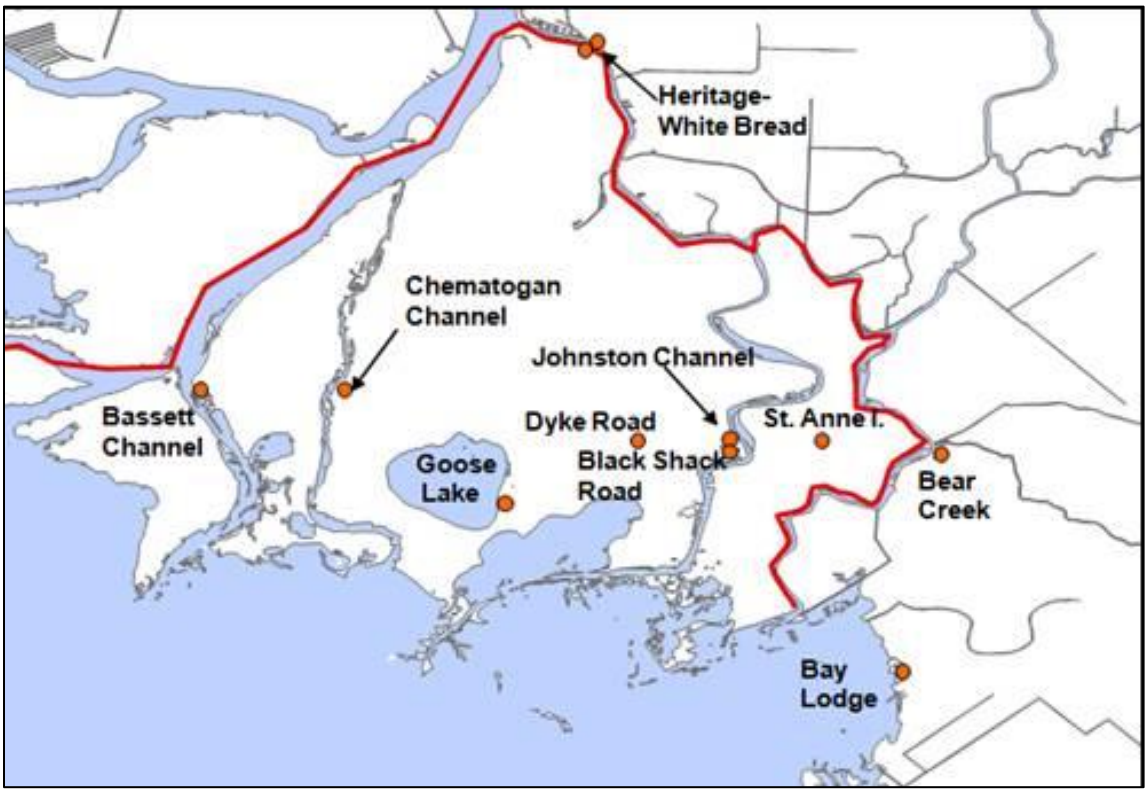


Figure 4. Study locations where northern leopard frogs were assessed for deformities and/or contaminant body burdens in 2006, 2007, 2011 and 2014.



Deformity rates are represented as a percentage of the total number of froglets collected at a site. For each survey, an effort was made to collect 100 frogs per site however this was not always possible so sample sizes ranged from 50-107 frogs per site in 2006, 42-135 frogs per site in 2007, 66-120 frogs per site in 2011 and 21-108 frogs per site in 2014.

Following 18 surveys at 10 AOC locations, deformity rates in froglets ranged from 0% to 11.6% over four study years (**Figure 5**). In 16 of 18 surveys, deformity rates were below the 5% threshold that is considered elevated in wild populations of amphibians (Oullett 2000). This threshold was exceeded in two surveys conducted at Chematogan Channel (11.6%) and Bay Lodge (6.7%) in 2007. However, deformity rates in subsequent surveys at Chematogan Channel in 2011 and 2014 and Bay Lodge in 2014 were well below this threshold, i.e., less than 1%. Therefore, elevated rates reported at these two locations in 2007 were not consistently found year-to-year and may have been episodic. Relative to other Great Lakes locations, deformity rates were intermediate in froglets from the St. Clair River AOC (mean=2.5%; based on 18 surveys) compared to rates reported in the Detroit River AOC (mean=5.9%; N=3 surveys) and Hamilton Harbour AOC (mean=1.0%; based on 4 surveys) and upstream Lake Huron reference sites (1.0%; based on 8 surveys) where similar surveys were conducted during this period (**Figure 6**).

Figure 5. Prevalence of deformities (%) in newly-transformed froglets from locations in the St. Clair River AOC and upstream Lake Huron reference sites in 2006, 2007, 2011, and 2014. The red line indicates the 5% threshold identified for deformities which is considered elevated in wild populations of amphibians (Ouellet 2000).

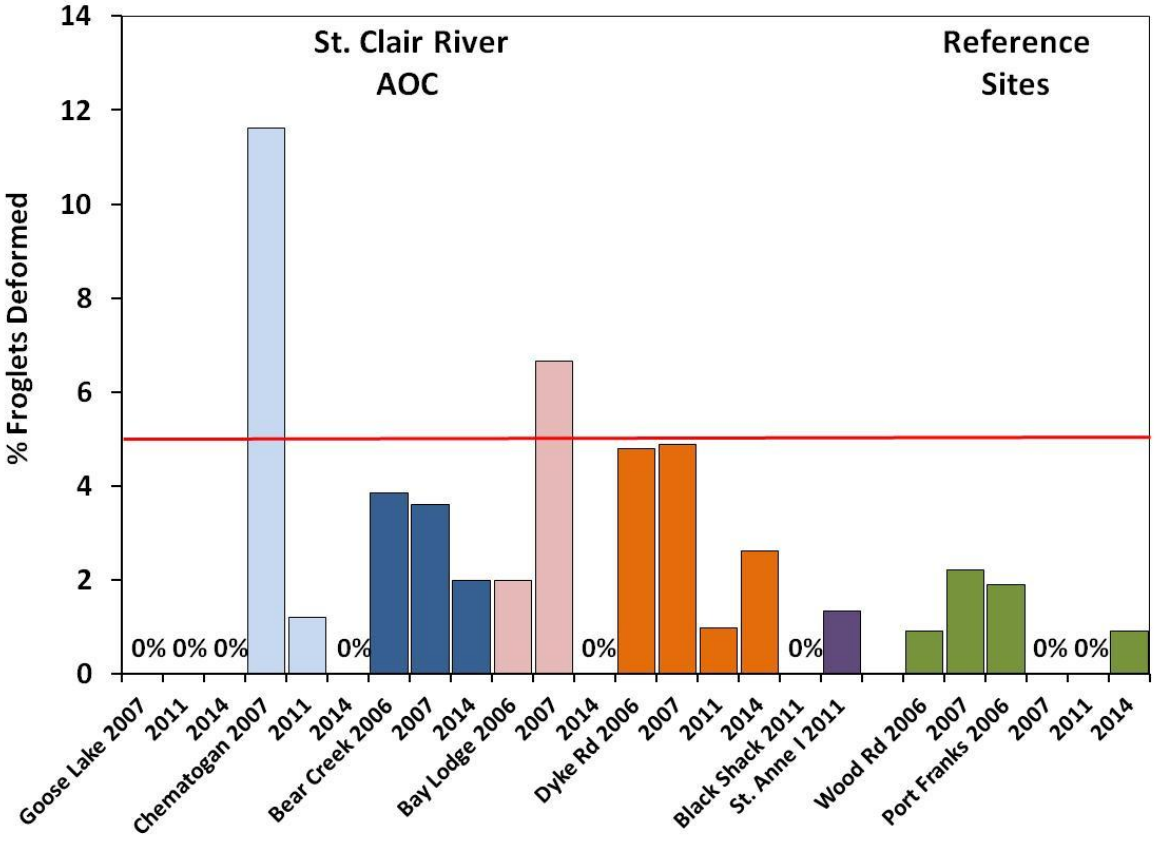
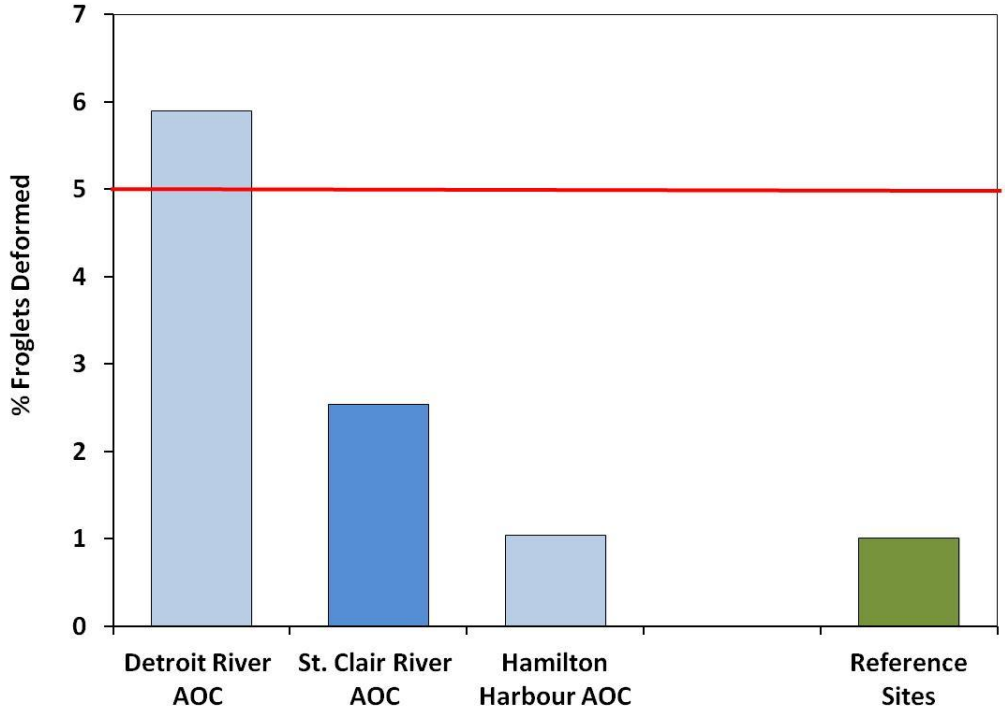


Figure 6. Prevalence of deformities (%) in newly-transformed froglets from multiple locations grouped together in the St. Clair River AOC, Detroit River AOC, Hamilton Harbour AOC and upstream Lake Huron reference sites following surveys conducted from 2006-2014. The red line indicates the 5% threshold identified for deformities which is considered elevated in wild populations of amphibians (Ouellet 2000).

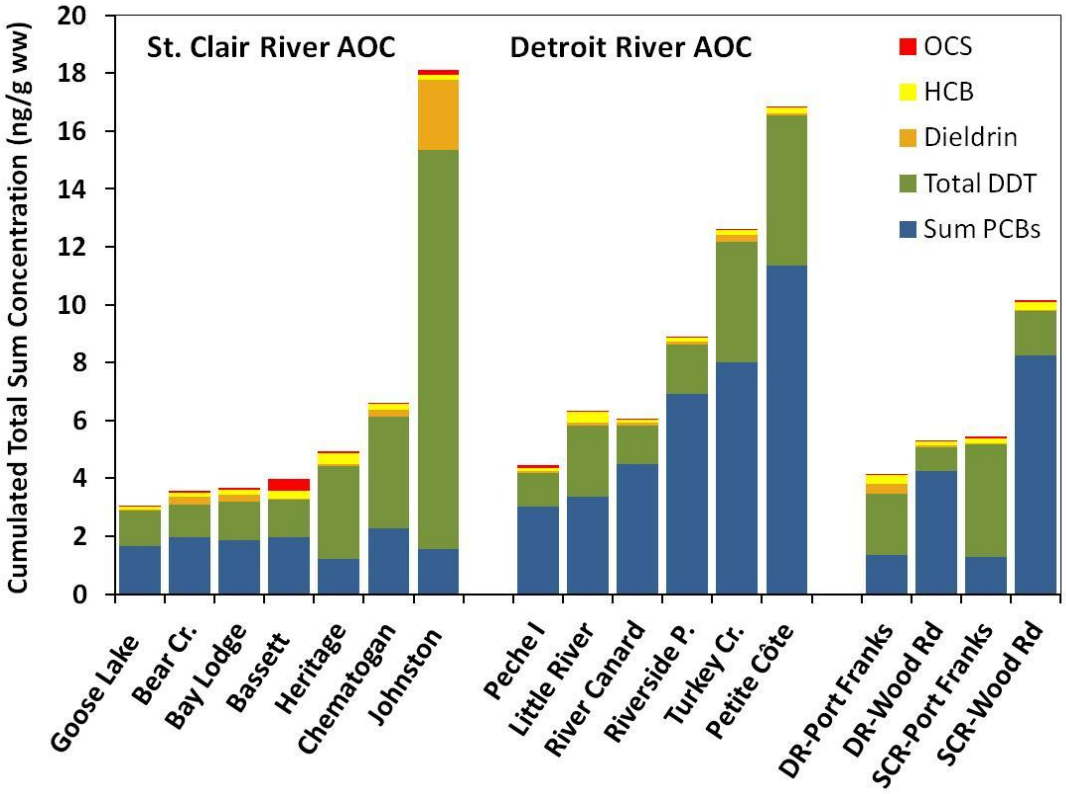


Contaminants in Northern Leopard Frogs

Young-of-year frogs were collected from seven St. Clair River AOC locations and two reference sites for an assessment of contaminant burdens in September or October of 2006 and 2007. Chemical analysis of whole bodies of frogs (minus gonads and spleens) were conducted at the National Wildlife Centre (NWRC) in Ottawa, Ontario and the Great Lakes Institute for Environmental Research (GLIER) at the University of Windsor, Ontario. Quantitative analysis of organochlorine compounds was performed using capillary gas chromatography using a mass selective detector (GC-MSD) operated in selected ion monitoring mode. For organochlorine analyses, frogs were analyzed as pools consisting of two same sex frogs with 5-7 pools of frogs analyzed per site in the two study years. Sum PCB concentrations were based on the sum of 34 or 62 individual or co-eluting PCB congeners. Chemical analysis for total mercury was analyzed using an Advanced Mercury Analyzer (AMA-254) equipped with an ASS-254 autosampler for solid samples according to CWS Method No. MET-CHEM-AA-03F. For mercury analysis, individual frogs (i.e., not as pools) were analyzed with nine or ten individuals per site. Organochlorine concentrations were reported on a wet weight basis and mercury concentrations were reported on a dry weight basis. Blanks, certified reference materials, and replicates for run for quality assurance purposes. Further details on chemical methods used are provided in Supplemental Information (Hughes *et al.* 2014).

Sum PCB concentrations in frogs were low with means generally ranging between 1-2 ng/g at the seven AOC locations (**Figure 7**). Total DDT concentrations, as the sum concentration of *p,p'*-DDE (1,1-bis-(4-chlorophenyl)-2,2-dichloroethene), *p,p'*-DDT, and *p,p'*-DDD (1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene), were largely similar to sum PCB concentrations with means ranging from 1-4 ng/g at study sites. One exception was for frogs from Johnston Channel where the mean concentration (13.8 ng/g) was relatively higher due elevated total DDT concentrations in two pools of frogs (25.3 ng/g and 18.9 ng/g). Frogs from Johnston Channel also had the highest mean concentration of dieldrin (2.4 ng/g) where the maximum concentration (8.0 ng/g) was also found. Mean dieldrin concentrations at other AOC locations were below 0.3 ng/g or were not detectable. Mean concentrations of HCB and OCS were less than 0.5 ng/g in frogs from all AOC locations. While some spatial differences were found in organochlorine burdens among study sites, these differences would not be expected to result in impacts on reproduction or development in AOC frogs. Current PCB body burdens in frogs from the AOC were below concentrations associated with toxic effects. PCBs in frogs from the AOC were four orders of magnitude lower than levels associated with increased mortality in wood frog (*Rana sylvatica*) tadpoles exposed to PCB-contaminated sediment (22000 ng/g; Savage *et al.* 2002). No evidence of toxicity was found in northern leopard frogs with a sum PCB body burden of 152 ng/g (Huang *et al.* 1999). Dieldrin concentrations in AOC frogs were well below the lowest observable

Figure 7. Cumulative total sum concentration (ng/g, wet weight) of mean OCS, HCB, dieldrin, total DDT and sum PCBs in northern leopard frogs from St. Clair River (SCR) AOC locations, Detroit River (DR) AOC locations and two upstream reference sites, Wood Road and Port Franks. Collection years were 2006 and 2007 for SCR locations, 2008 and 2009 for DR locations, and all four years for reference sites. Each location is ranked from the least contaminated to the most contaminated.

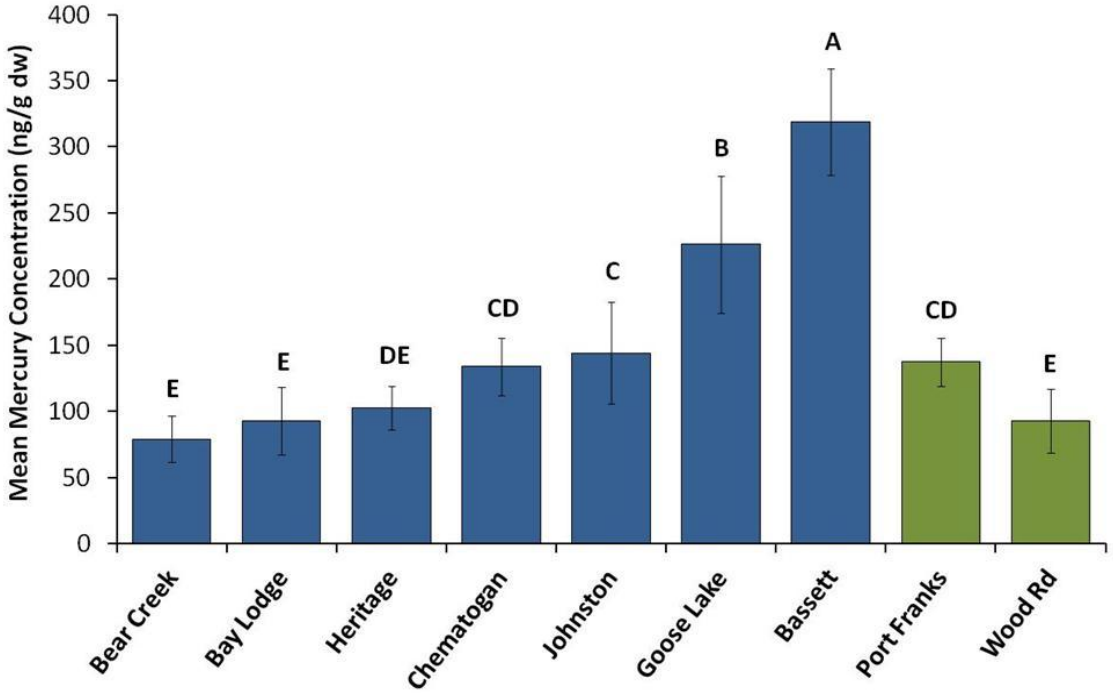


effect level of 600 ng/g in northern leopard frog tadpoles exposed to dieldrin in water for 28 days (Schuytema *et al.* 1991). In addition, total DDT concentrations in frogs from the AOC (including from Johnston Channel) were at least two orders of magnitude less than the lowest reported effect concentrations of 2400 ng/g for DDT in common frog (*R. temporaria*) tadpoles (Cooke 1972).

Overall, mean cumulative total sum concentrations of OCS, HCB, dieldrin, total DDT, and sum PCBs in whole bodies of frogs from the St. Clair River AOC were generally lower than concentrations in frogs from the Detroit River in 2008 and 2009 and more comparable to concentrations in frogs at the Lake Huron reference sites (**Figure 7**). Differences in cumulative concentrations in frogs between the AOCs for these five compounds are largely driven by differences in PCB concentrations which were notably higher in the Detroit River AOC.

Concentrations of mercury varied significantly among study sites with means ranging from 79 ng/g in frogs from Bear Creek to 319 ng/g in frogs from Bassett Channel (**Figure 8**). Frogs from two AOC locations, Bassett Channel and Goose Lake, had significantly higher mercury burdens compared to frogs from the two upstream Lake Huron reference sites while frogs from the other five AOC locations had burdens were similar to those from the reference sites. Bassett Channel is located at the bottom of the St. Clair River and downstream from historical industrial point sources of mercury pollution in the AOC.

Figure 8. Mean concentrations (SD, ng/g, dry weight) of total mercury in northern leopard frogs collected from seven St. Clair River AOC locations and two upstream reference sites, Port Franks and Wood Road (green), in 2006 and 2007. Means sharing the same uppercase letters are not significantly different.



However, relatively higher mercury levels in frogs from Goose Lake are unexpected since this lake is remote from these sources and water entering the lake from the Dredge Cut waterway is currently controlled by a pumps and weirs. Consistent with this, mercury in sediment collected from Goose Lake in 2005 did not exceed Ontario sediment quality guidelines (Great Lakes Institute for Environmental Research 2008). It is possible that young-of-year frogs collected from this site may have been recent arrivals to this area and have hatched as eggs and developed as tadpoles in another nearby water body.

Relative to other vertebrates, few studies have examined bioaccumulation and effects of mercury in amphibians. One laboratory dosing study showed that total mercury body burdens of 240 and 400 ng/g dw in southern leopard frog (*Rana sphenocephala*) tadpoles were associated with adverse effects including increased mortality, malformations, and increased time to tail resorption (Unrine *et al.* 2004). Young-of-year frogs from Bassett and Goose Lake had body burdens that were within the range associated with these effects. Other studies however have shown no effects in amphibians associated with these mercury burdens (Bergeron *et al.* 2011; Wada *et al.* 2011). It is also important to note that there are differences in the sensitivity to mercury among species and the relative sensitivity of northern leopard frogs to mercury is not known. Overall, mercury concentrations in AOC frogs (overall mean=156 ng/g) were lower than concentrations in other amphibian species from other sites historically contaminated with mercury and were generally comparable to concentrations at either sites upstream of these point sources or remote sites, e.g., Acadia National Park, where atmospheric deposition represents the major route for mercury input (Bank *et al.* 2007; Bergeron *et al.* 2010; Burke *et al.* 2010; Loftin *et al.* 2012). It is not clear if adverse effects would be found in frogs from Bassett Channel and Goose Lake where the highest mercury burdens were found.

4. Snapping Turtle Study

Snapping turtle eggs were collected from 41 locations in the Walpole Island delta (Walpole Island First Nation) in the St. Clair River AOC in May of 2011 (**Figure 9**). Collection locations covered a wide geographic area within the delta. Entire clutches of eggs were collected within 48 hours of oviposition, placed in plastic containers containing moistened vermiculite (**Figure 10**) and artificially incubated at approximately 18-24^{o}C in a laboratory at the Canada Centre for Inland Waters (CCIW) in Burlington, Ontario until the time of hatching. Hatchling turtles were visually scored for gross morphological deformities of the shell, eyes, head, limbs, and tail. Hatching success were calculated for each clutch as the number of eggs hatched relative to the number of eggs incubated and rates of deformities were calculated as the number of deformed hatchlings relative to the number of eggs hatched. At the time of collection for 37 of 41 clutches, approximately five eggs per clutch were randomly selected and sent off for contaminant analysis. The non-AOC reference site selected for this assessment was Tiny Marsh located near Elmvale and inland of Lake Huron (44°36'05.50”N, 79°56'09.39”W) where eggs were collected from 2001-2003. Eggs collected in 2002 and 2003 from Tiny Marsh were artificially incubated in the laboratory under identical conditions and the results for the two endpoints were used for comparison here. Contaminant burdens are compared to other Great Lakes sites (including AOCs) where similar assessments were conducted from 2001-2004 and are provided for context. At the completion of the incubation study, hatchling turtles were released back into the delta at the specific collection location from which the clutch of eggs had been collected.

Figure 9. Collection locations of snapping turtle clutches in the Walpole Island delta in the St. Clair River AOC in 2011. Locations are shown with clutch identifications designated from W1 through to W41. Four clutches, W12, W13, W14, and W15, were not analyzed for contaminants.

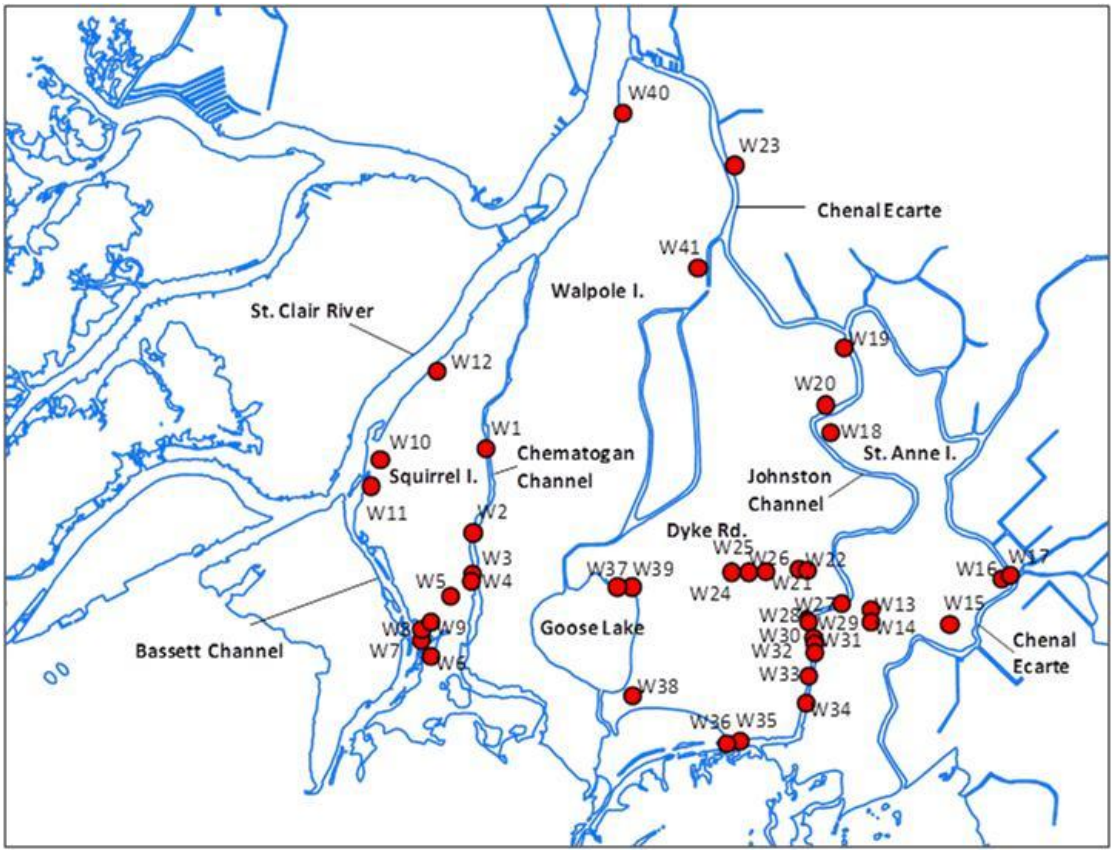
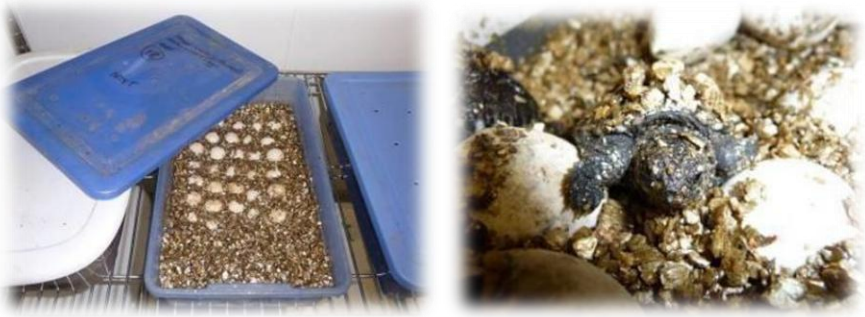


Figure 10. Snapping turtle eggs incubated in the CCIW lab and snapping turtle hatchling.



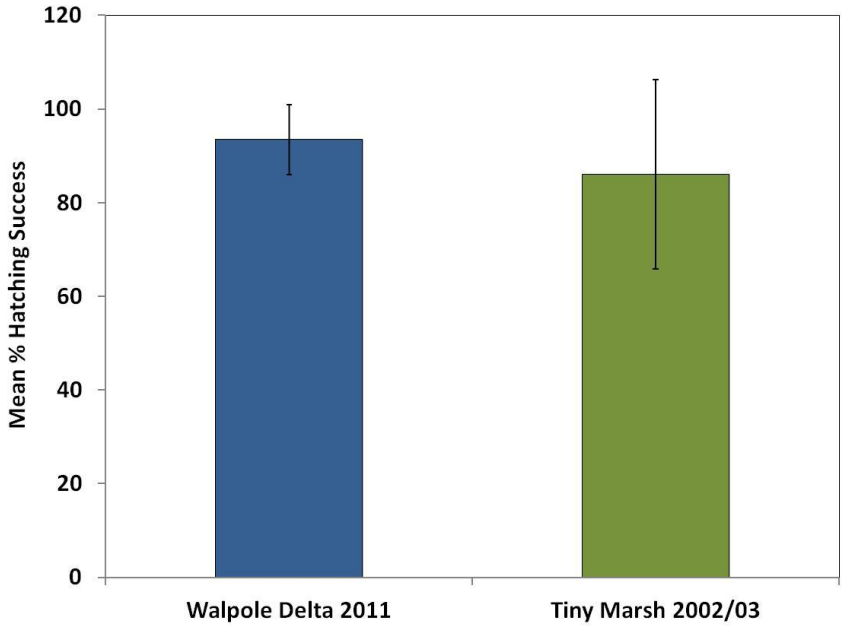
Hatching Success & Hatchling Deformities

Mean hatching success of snapping turtle eggs collected from the Walpole Island delta in 2011 was high at 93.5% in comparison to mean hatching success in eggs from the upstream Lake Huron reference site in 2002 and 2003 (86.0%, based on 20 clutches; **Figure 11a**). No significant difference in hatching success was found between clutches from the AOC and the inland of Lake Huron reference site at Tiny Marsh.

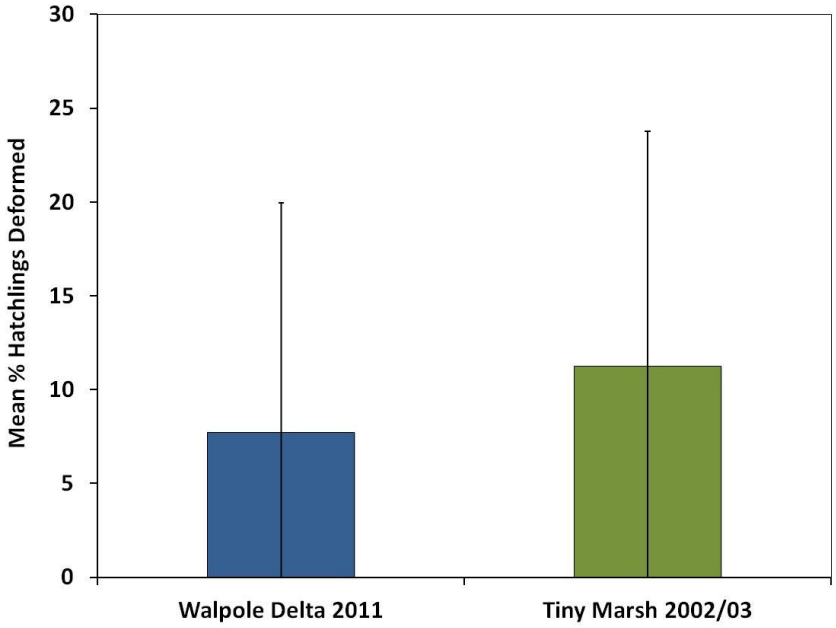
The mean percentage of deformed snapping turtle hatchlings from the Walpole Island delta was 7.7% in comparison to the Tiny Marsh reference site where the mean was relatively higher at 11.3% in 2002 and 2003 (based on 20 clutches; **Figure 11b**). Similarly, there was no significant difference in mean deformity rates between hatchlings from the AOC and those from the inland of Lake Huron reference site.

Figure 11. Mean hatching success (SD) of snapping turtle eggs (a) and mean percentage (SD) of deformed hatchlings (b) from Walpole Island delta in the St. Clair River AOC in 2011 and inland of Lake Huron reference site, Tiny Marsh, in 2002 and 2003.

a)



b)



Consistent with the pattern shown here using Tiny Marsh as the reference site, no significant differences were found for either of these endpoints when Walpole Island delta eggs were compared to clutches of eggs collected in 2011 from Algonquin Provincial Park, a site remote from the Great Lakes (mean hatching success=96.2% and mean percentage of deformed hatchling turtles=8.9%, based on 12 clutches; Hughes *et al.* 2015). Overall, hatching success was among the highest and rates of deformed hatchlings were among the lowest in turtles from the AOC in 2011 compared to rates reported at other Great Lakes sites in similar artificial incubation studies conducted in 2002-2004 (de Solla *et al.* 2007; Hughes *et al.* 2015).

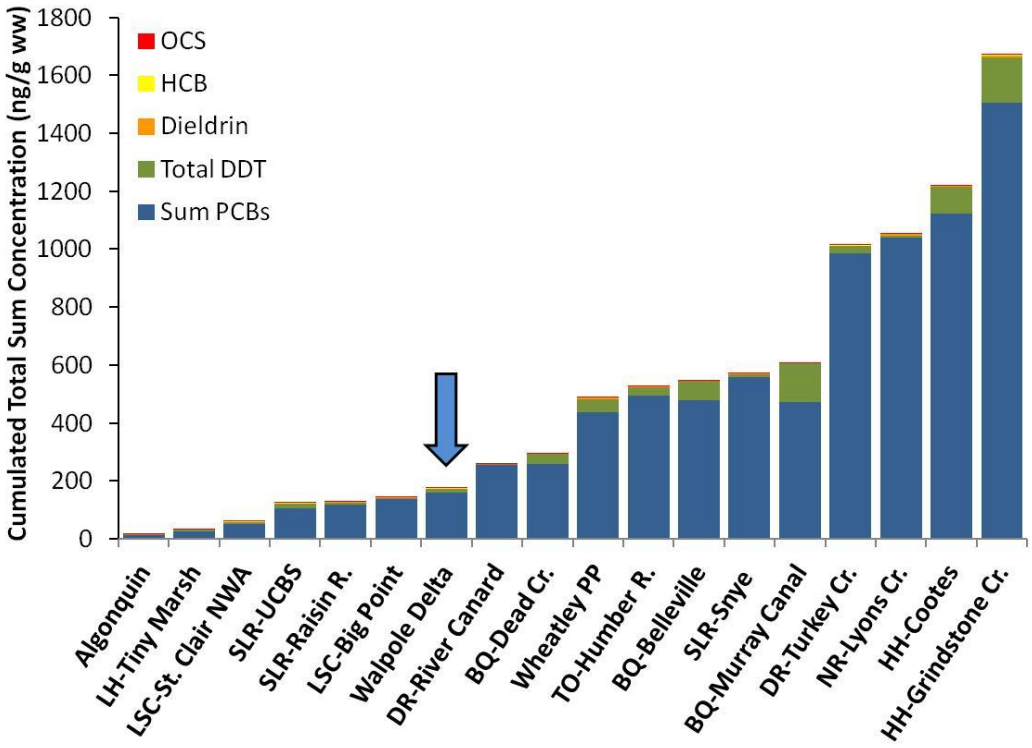
Contaminant in Snapping Turtle Eggs

Eggs were shipped frozen to GLIER and 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 39 individual and co-eluting PCB congeners. Eggs were analyzed for total mercury at NWRC using a Direct Mercury Analyzer (DMA-80). Organochlorine concentrations were reported on a wet weight basis and mercury concentrations were reported on a dry weight basis. Blanks, certified reference materials, and replicates for run for quality assurance purposes. Further details on chemical methods used are provided in Supplemental Information (Hughes *et al.* 2015).

Sum PCBs in snapping turtle eggs collected from the Walpole Island delta in 2011 ranged widely from 7.8 ng/g to 725.7 ng/g and were found at the highest concentrations (mean=175.2 ng/g) compared to all other organochlorines. Mean concentrations of total DDT (11 ng/g), dieldrin and OCS (both 2 ng/g) and HCB (1 ng/g) were relatively lower. Significantly higher concentrations of sum PCBs, OCS, HCB and dieldrin were found between eggs from Walpole Island delta and those collected from the reference site at Tiny Marsh in 2001-2003 while total DDT concentrations were not significantly different between the two sites.

A Great Lakes perspective of mean cumulative total sum concentrations of OCS, HCB, dieldrin, total DDT, and sum PCBs in snapping turtle eggs from Walpole Island delta in 2011 and other Great Lakes sites from 2001-2004 is provided in **Figure 12**. Overall, turtle eggs from Walpole Island delta ranked 7th of 18 Great Lakes sites, from lowest to highest total burdens, where eggs were collected during this earlier time period. Organochlorine burdens in eggs collected in 2001 from two Lake St. Clair sites, Big Point Hunt Club and the St. Clair NWA, and situated approximately 13 kilometres south of the St. Clair River AOC boundary are shown. No significant differences were found in concentrations of the five organochlorines between eggs collected from the St. Clair River AOC and eggs from the two downstream Lake St. Clair sites (combined; Hughes *et al*. 2015). Overall, eggs from other AOCs including Hamilton Harbour, Bay of Quinte and Detroit River had relatively higher organochlorine burdens compared to the St. Clair River AOC. Eggs collected from a contaminated area on the Upper Hudson River in New York had much higher sum PCB concentrations (mean=3953 ng/g) that was associated with a 60% mortality rate in juvenile turtles at 14 months of age compared to a 10% mortality rate in juveniles from the reference area where sum PCBs in eggs were also substantially lower (mean=61 ng/g; Eisenreich *et al*. 2009).

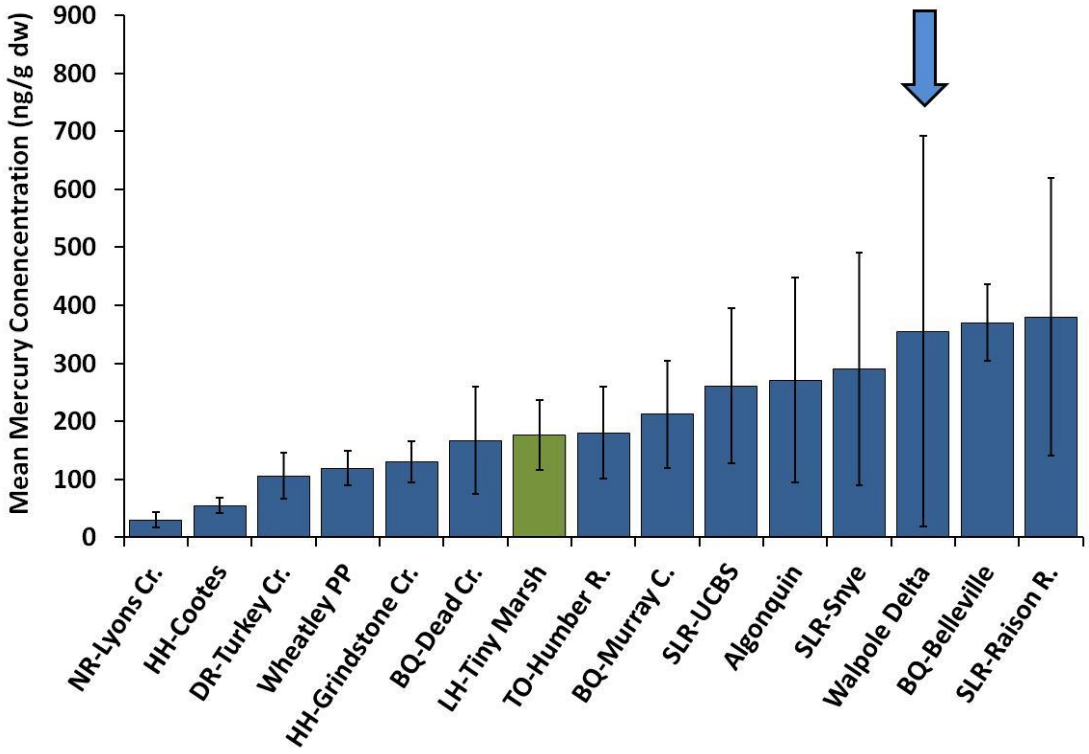
Figure 12. Mean cumulated total sum concentration (ng/g, wet weight) of OCS, HCB, dieldrin, total DDT, and sum PCBs in snapping turtle eggs from Walpole Island delta in 2011 (arrow) and various sites in the Great Lakes basin including AOCs, Tiny Marsh, Lake St. Clair (LSC), and Algonquin Provincial Park (remote site) in 2001-2004.



Total mercury concentrations ranged widely in Walpole Island delta eggs with a mean concentration of 355.1 ng/g (range=10.6 ng/g-1011.8 ng/g, dry weight). Mercury burdens were relatively lower in eggs from the Tiny Marsh reference site in 2001-2003 (mean=175.8 ng/g). There was no significant difference in mercury concentrations between the two sites; however, this is likely explained by the large variability in mercury concentrations in clutches among AOC locations. There was no apparent spatial pattern for mercury burdens among collection locations of eggs throughout the delta. Evidence based on stable isotope analysis suggests that turtles are feeding on varying types of prey around the delta including those from different trophic levels, a finding that is consistent with the opportunistic nature of this species. In addition, higher mercury exposure is related to turtles feeding on higher trophic level prey types. This also contributes to variability in mercury concentrations found in turtle eggs in Walpole Island delta.

A Great Lakes perspective of mean mercury concentrations in snapping turtle eggs from Walpole Island delta in 2011 and other Great Lakes sites from 2001-2004 is provided in **Figure 13**. Overall, turtle eggs from Walpole Island delta ranked 13th of 15 Great Lakes sites, from lowest to highest mercury burdens, where eggs were collected during this earlier time period (**Figure 13**). Mercury concentrations in eggs collected from Algonquin Provincial Park from 2002-2004 were intermediate to concentrations at Walpole Island delta and Tiny Marsh (mean=271.2 ng/g, based on 12 clutches).

Figure 13. Mean mercury concentrations (ng/g, dry weight) in snapping turtle eggs from Walpole Island delta in 2011 (arrow) and various sites in the Great Lakes basin including AOCs, Tiny Marsh (green), Lake St. Clair (LSC), and Algonquin Provincial Park in 2001-2004.



Relative to other biota (e.g., birds), few studies have examined 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 3000 ng/g dw in eggs from a mercury-contaminated study site in Virginia was associated with a 12% reduction in hatching success relative to the reference sites. Eggs from Walpole Island delta had a mean mercury concentration that was approximately one-eighth of this concentration and that was in fact more comparable to concentrations in eggs from the reference sites in that study (estimated range=35-100 ng/g dw). This is consistent with high hatching success (93.5%) reported in AOC turtles in this study where contaminant burdens in eggs from the same clutch were also measured.

5. Conclusions

Hatching success and deformity rates were the two biological endpoints used to assess the status of BU #5 - *Bird or Animal Deformities or Reproduction Problems* - in two sentinel wildlife species in the St. Clair River AOC. Hatching success and deformities were assessed at two important developmental stages in northern leopard frogs in the laboratory and in the wild. Exposure studies were conducted on fertilized eggs exposed to water (2007) and water and sediment (2011) from several AOC locations to assess the impact of exposure of these media on egg hatchability and amphibian development. The results of these

studies indicated that mean hatching success of leopard frog eggs was high (>98%), mean percentages of embryos with a deformity were low to moderate (<7%) and rates of these two endpoints were statistically similar to rates reported at non-AOC reference sites. Surveys of wild populations of frogs indicated that deformity rates in froglets were low overall and below the 5% threshold based in 16 of 18 surveys conducted over a four year period within the AOC. In two surveys conducted in 2007 where the threshold was exceeded, no evidence of elevated deformity rates was found at these two locations in 2011 and/or 2014. Therefore, exceedences reported above 5% at these two sites in 2007 were not consistently found in later years.

Snapping turtle eggs collected from many locations throughout Walpole Island delta were artificially incubated in the laboratory to assess hatching success of eggs and deformity rates of hatchlings. Consistent with the results found for leopard frogs, mean hatching success of snapping turtle eggs was high (93.5%), the mean percentage of deformed hatchlings was low (7.7%) and no significant differences were found for either of these endpoints compared to rates found at the non-AOC reference site.

Contaminant burdens in sentinel wildlife were used to support the results reported for these biological endpoints. Comparisons to burdens reported for these species at other Great Lakes sites provide some context and help in ascertaining the influence of historical local AOC contaminant sources, the effects of which may have diminished with time in wildlife following restrictions in use and production of these compounds and successful remediation at an AOC. Comparisons to contaminant concentrations associated with effects on reproduction and development and toxicity in the scientific literature are also informative. Overall, organochlorine burdens in frogs for four compounds of concern in the AOC, i.e., PCBs, dieldrin, HCB and OCS, and as well as the pesticide DDT (with its breakdown products), were generally lower than frogs in the Detroit River AOC and were more similar to concentrations in frogs from the Lake Huron reference sites. Sum PCBs, dieldrin and total DDT concentrations in wild frogs from the AOC were well below those associated with toxicity. Similarly, organochlorine burdens in snapping turtle eggs were ranked as intermediate relative to eggs from other Great Lakes sites including other AOCs. Concentrations of mercury, as another contaminant of concern, varied among St. Clair River AOC locations in frogs and turtle eggs and potential effects associated with the highest levels of exposure are not known. Mercury concentrations in frogs and turtles were not notably elevated relative to other sites with a history of local point sources of mercury pollution. Based on the criteria used to assess wildlife reproduction and deformities in these studies, there was no evidence of impairment for these biological endpoints in two aquatic wildlife sentinel species in the St. Clair River AOC (Ontario).

6. Recommendation for Status Change

The results of these studies provide sufficient evidence to support a recommendation to deem the status of the BU #5 - *Bird or Animal Deformities or Reproduction - a*s “Not Impaired” in the St. Clair River Area of Concern (Ontario).

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8. Supplemental Information