DETROIT RIVER AREA OF CONCERN (ONTARIO)

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

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Executive Summary

Studies were conducted by Environment and Climate Change Canada to assess whether there is evidence of impaired reproduction in wildlife resulting from elevated contaminant exposure in the Detroit River Area of Concern (AOC) in Ontario, Canada. This work is directly relevant to the current assessment of Beneficial Use Impairment (BUI) #5 - *Bird or Animal Deformities or Reproduction Problems*. Several sentinel wildlife species with different important attributes (e.g., trophic position, food sources), that are sensitive to the effects of contaminants, and with close connections to the aquatic environment were selected. Measures of reproduction were assessed against delisting criteria or environmental targets defined for the AOC. Studies were conducted in the laboratory where eggs were incubated under controlled conditions to assess whether intrinsic factors such as contaminants may induce early mortality of embryos. Studies were also conducted in the field where other stressors that impair reproduction may be important. Reproduction parameters were compared to background levels at reference sites to elucidate the influence of AOC-localized effects. Legacy contaminants that included polychlorinated biphenyls (PCBs) and mercury were measured in tissues and concentrations were compared to threshold concentrations associated with impairment of reproduction.

Embryonic viability in double-crested cormorants (*Phalacrocorax auritus*) and hatching success of common snapping turtle (*Chelydra serpentina*) eggs were similar between incubated eggs collected in the AOC and those from respective reference sites in all years of study. Hatching success of northern leopard frog (*Rana [=Lithobates] pipiens*) eggs was similar between AOC and reference sites following exposure of eggs to water, and to water and sediment collected from several locations with varying degrees of contamination, on the River. In addition to successful hatching of eggs, the ability of nestlings to grow and successfully fledge (productivity) from the nest is also a critical measure of breeding success. Field studies of nesting tree swallows (*Tachycineta bicolor*) and black-crowned night-heron (*Nycticorax nycticorax*) indicated that hatching success and productivity of birds at sites in the Detroit River AOC were similar to those from upstream reference sites in five of six study years. The single exception was for night-heron at the AOC colony where reduced reproductive success in 2009 was likely due to extrinsic factors (e.g., predation) and not contaminant-induced effects in that year. Productivity estimates for herring gulls (*Larus argentatus*) at two surrogate AOC colonies in Lake Erie, immediately downstream and under the influence of conditions in the AOC, were sufficiently high to maintain a stable gull population. For the large majority of samples, concentrations of PCBs and mercury in frogs (whole bodies), turtle (eggs) and birds (eggs and nestling liver) were below thresholds known to result in adverse effects on reproduction in the AOC. In a few cases, egg samples for tree swallows and night-heron exceeded reproduction thresholds for mercury; however, hatching success and fledging success were not reduced in these years. Based on criteria used to assess reproduction in wildlife studies conducted from 2008–2019, there was no evidence to suggest that exposure to legacy contaminants originating from localized sources in the AOC resulted in impaired reproduction in sentinel species in the Detroit River AOC (Ontario).

The results of these studies provide sufficient evidence to support a recommendation to deem the status of the BUI #5 - *Bird or Animal Deformities or Reproduction* - as "*Not Impaired*" in the Detroit River Area of Concern (Ontario).

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1. Introduction

The Detroit River is an important waterway on the Great Lakes that is shared by Canada and the United States and connects Lake St. Clair to Lake Erie. The River provides water for industries and large municipalities, has important recreational uses for the public, and is critical to the shipping industry for transportation of goods on the Great Lakes and globally. The fast-flowing and largely ice-free conditions of the River also provide key habitat to many resident and migratory birds year-round. Unfortunately, decades of misuse of this resource over the last century resulted in local degradation of the aquatic environment that impaired its use for people, fish, and wildlife. Historical discharges of pollutants from industries, municipal wastewater plants, combined sewer overflows, and urban and rural runoff contributed to impaired water quality and degraded sediment along both sides of the River (Michigan Department of Natural Resources (MDNR) and Ontario Ministry of the Environment (OMOE) 1991). Consumption advisories were issued following high levels of polychlorinated biphenyls (PCBs) and mercury reported in fish (MDNR and OMOE 1991). Significant loss and modification of historic natural features along the shoreline and in the watershed resulted in loss and/or degradation of critical habitat required for fish and wildlife (MDNR and OMOE 1991). This contributed further to poor water quality in the Detroit River. Environmental regulations limiting discharges of pollutants by industries and municipalities that had been implemented for decades on both sides of the Detroit River were not sufficient to remedy these issues alone.

These conditions and pressures were not unique to the Detroit River but were evident at other populated and industrialized sites on the Great Lakes. In 1987, the Detroit River was designated along with 42 other sites within the Great Lakes basin as an "Area of Concern" (AOC) since 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*." Fourteen beneficial use impairments (BUIs) were assessed under the GLWQA to identify environmental problems that have impaired use of the aquatic ecosystem and thereby guide restoration activities. One of these is BUI #5 - *Bird or Animal Deformities or Reproduction Problems* – that addresses contaminant exposure or other anthropogenic environmental stressors that may adversely impact reproduction or development of wildlife. In accordance with the provisions of the GLWQA, a Remedial Action Plan (RAP) was developed and 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 BUIs determined to be impaired; and the final stage is when all impairments are restored.

The official designation (status) of BUI #5 has changed through the course of this iterative process as wildlife studies were conducted and reviewed and details for assessment were refined. The *1991 Stage 1 Report* for the Detroit River concluded that the status of BUI #5 was *Not Impaired* following no evidence of reproductive problems or bird or animal deformities in the AOC (MDNR and OMOE 1991). This was based on reports of substantial and diverse populations of colonial waterbirds and waterfowl in the AOC that also coincided with significant reductions in environmental contaminants in the Great Lakes basin since the 1970s. In addition, no known cases of bird or animal deformities were documented. In 1996, following reproductive failure of bald eagles (*Haliaeetus leucocephalus*) on the

Canadian shoreline in some years, it was recommended that the status of BUI #5 be changed to *Requires Further Assessment* (*RFA*) due to insufficient information available (Michigan Department of Environmental Quality (MDEQ) 1996). Since 1996, the implementation of the RAP has functioned separately on the Canadian and American sides of the Detroit River. In 1999, there was formal agreement by the Detroit River Canadian Cleanup Committee - who is responsible for implementation of the RAP on the Canadian side of the River - to change the status of BUI #5 to *Impaired* (Detroit River Canadian Cleanup Committee 1999).

Subsequent studies conducted in the early 2000s by Environment and Climate Change Canada (ECCC) suggested continued evidence of reproductive impairment in aquatic-feeding wildlife on the Detroit River AOC (Ontario) that might be associated with exposure to local sources of contaminants (Environment Canada 2010). In 2001, herring gulls (*Larus argentatus*) from a colony on Fighting Island in the Detroit River AOC had reduced egg viability that was consistent with possible chemical-mediated embryonic mortality. There was also a significant sex ratio bias toward more male herring gull chicks at this colony compared to the balanced sex ratio reported at the reference colony. Vitellogenin, a protein normally found in egg-producing females, was detected in 20% of adult males (2/10 gulls) from this colony in 2002. These trends were not consistently found in all study years. For the common snapping turtle (*Chelydra serpentina serpentina*), hatching success was significantly reduced in eggs collected from sites on Turkey Creek in the AOC relative to reference sites in 2002–2004 (de Solla *et al.* 2008). Despite dramatic declines in organochlorine contaminants in herring gull eggs in the AOC since the late 1970s, contaminant burdens, notably PCBs, were found to be substantially higher in eggs of gulls and turtles in the Detroit River AOC compared to reference sites (de Solla *et al.* 2007; Environment Canada 2010). Following this work, it was recommended in 2006 that the status of this BUI remains *Impaired* (Leney and Haffner 2006) and in 2010, this change was officially adopted in the *Stage 2 Report* (Green *et al.* 2010).

Numerous Detroit River RAP projects have been completed by the Detroit River Canadian Cleanup (DRCC) stakeholders and significant progress has been made toward the delisting process for this AOC (Green *et al.* 2010). Several years had past since the last BUI #5 assessment and a current assessment was required. This status recommendation report summarizes the results of several studies conducted from 2008–2019 by ECCC that examined reproduction of wildlife on the Ontario side of the Detroit River and provides a recommendation for the current status of BUI #5 in the Detroit River AOC.

2. Assessment Approach

Consistent with guidance provided by the International Joint Commission (IJC), sentinel wildlife species were selected for assessment of this BUI (IJC 1991). All six sentinel species selected meet the same basic requirements as good bio-indicators of environmental contamination conditions in the AOC: they generally have high bioaccumulation rates of persistent organic pollutants; they are sensitive (to some degree) to the effects of contaminants that may impair their ability to reproduce successfully; and they have close connections to the aquatic environment. These sentinel species each also have their own merits. Colonial waterbirds including the black-crowned night-heron (*Nycticorax nycticorax*), double-crested cormorant (*Phalacrocorax auritus*), and herring gull are ideal biological indicator species since they have predominately fish-based diets and feed at the top of the food chain. This means that they are likely to have the highest contaminant burdens in their tissues – and thereby potentially show adverse effects associated with exposure to these compounds - relative to lower trophic level species. The snapping turtle is a semi-aquatic top predator that is non-migratory with a small home range making it a good bioindicator of local contamination. Tree swallows (*Tachycineta bicolor*) are a mid-trophic level wildlife species that feed on benthic insects that emerge as adults from waterbodies. Contaminated sediment at sites along the River has been identified as contributing to toxic effects in aquatic organisms (Green *et al.* 2010) and tree swallows are ideal since contaminants found in eggs and nestlings are associated with the degree of sediment contamination near their nest boxes (Custer *et al.* 2016, 2017). Northern leopard frogs (*Rana pipiens*) spend part of their life in the water and feed on aquatic biota both as tadpoles and as frogs. Although amphibians have lower dietary bioaccumlation rates than birds, they can also absorb contaminants through their skin, and during their juvenile (tadpole) stage, through their gills. Their eggs are also sensitive to effects of poor water quality for hatching and proper development. The utility of these species, and general ease of collections for monitoring, support their use as bioindicators in similar wildllife assessments by ECCC and others conducted at other Great Lakes AOCs (e.g., Custer *et al.* 2018).

Sentinel species, years of study, and reports providing details of five ECCC studies assessing reproduction in the AOC are as follows and are provided in the Appendix:

i. Northern leopard frogs, 2008–2013; *Reproductive Health and Development in Northern Leopard Frogs (Rana pipiens) in the Detroit River Area of Concern (Ontario)*

ii. Black-crowned night-herons, 2009 & 2011; *An Assessment of Breeding Success of Black-crowned Night-Herons (Nycticorax nycticorax) in the Detroit River Area of Concern (Ontario)*

iii. Snapping turtles, 2014–2016; *Assessment of the Wildlife Reproduction and Deformities Beneficial Use Impairment in the Detroit River Area of Concern (Ontario) – Snapping Turtles*

iv. Other colonial waterbirds, 2015–2019; *Assessment of the Wildlife Reproduction and Deformities Beneficial Use Impairment in the Detroit River Area of Concern (Ontario) – Colonial Waterbirds*

v. Tree swallows, 2016–2019; *Assessment of the Wildlife Reproduction and Deformities Beneficial Use Impairment in the Detroit River Area of Concern (Ontario) – Tree Swallows*

This status re-designation report provides a brief overview of the above studies and highlights the findings that pertain to the assessment of BUI #5 in the St. Detroit River AOC (Ontario). Since there was no evidence of bird deformities reported in the AOC, the assessment of BUI #5 was revised to focus on reproduction only (Green *et al.* 2010). Delisting criteria were established to assess the status of BUI #5 in the AOC (Green *et al.* 2010). Delisting criteria are environmental targets against which the success of remediation and restoration efforts in AOC are measured. Specifically, BUI #5 will no longer be impaired when conditions for two criteria are met:

Criterion 1: *When incidence rates of bird and animal reproductive problems in sentinel wildlife species do not exceed background levels at suitable reference sites elsewhere in the Great Lakes basin or suitable inland control populations for a minimum of three years; and*

Criterion 2: *When scientifically defensible wildlife bioassays of indicator species confirm that there are no reproductive problems and no significant toxicity from the water column or sediment contaminants or bioaccumulation.*

With regard to criterion 1, reproduction in sentinel species was assessed both in the laboratory and in the field. Artifical incubation of eggs in the lab, such as was done in turtles and cormorants, assesses embryonic viability due to intrinsic factors (e.g., contaminants) that may induce mortality and potentially result in population-level effects. The potential for reproductive impairment in amphibians was examined by exposing leopard frog eggs to water (2008) and water and sediment (2010) collected from multiple locations in the AOC and then assessing the ability of these eggs to hatch and develop normally. Field studies of breeding black-crowned night-heron, herring gulls, and tree swallows at sites along the River were conducted to assess whether these populations were able to reproduce successfully. All species were monitored for three years at a minimum with the exception of the black-crowned night heron study that was conducted over two years. It is important to note that other parameters associated with growth and development, biochemical changes potentially associated with contaminant exposure, and/or measures of overall health were also examined in these studies; details of these findings are provided in the reports above.

With regard to criterion 2, this refers to the accumulation of contaminants in tissues above a threshold concentration at which an adverse impact on reproduction might be expected. Two legacy contaminants, PCBs and mercury, that have contributed to the history of environmental degradation in the Detroit River AOC are reported on in wildlife here. Concentrations in tissues are assessed against threshold concentrations above which an impairment on reproduction in birds, turtles, and amphibians might be expected. Where these were not available in the scientific literature, thresholds associated with toxicity are used. Chemical analyses of tissues were conducted at accredited laboratories at the National Wildlife Centre (NWRC) in Ottawa, Ontario or at the Great Lakes Institute for Environmental Research (GLIER) at the University of Windsor, Ontario. Sum PCB concentrations are based on the sum of 34 or 35 individual or co-eluting PCB congeners unless noted otherwise. Contaminant concentrations are reported on a wet weight basis unless noted otherwise. In addition to other legacy compounds (e.g., DDT), contaminants of emerging concern (e.g., brominated flame retardants, perfluorinated compounds) were also quantified in tissues in these studies. Further details of chemical analyses and concentrations of these compounds are provided in the respective reports. Statistical significance is determined as $p \leq 0.05$ for all statistical analyses.

3. Northern Leopard Frogs Studies

Frog Embryonic Exposure Studies

A critical first step for an assessment of amphibian reproduction in an area is that viable eggs deposited at a site can successfully hatch and develop into free swimming tadpoles. To examine hatching success of frog eggs, embryonic exposure studies were conducted under controlled laboratory conditions for two different scenarios. To assess the impact of AOC water quality on amphibian hatching success, northern leopard frog eggs were raised in water collected from seven locations along the Detroit River in 2008 (**Figure 1**). Since contaminated sediment can also be a significant source of contaminants to amphibians, another exposure study was conducted in 2010 in which eggs were raised in water and sediment collected from an additional five Detroit River locations that included three sites on the American side of the River (**Figure 1**). This is an adaptation of the standard FETAX test which is used to assess water quality for both acute toxicity; therefore, this test is ideal for the assessment of reproductive impairment following exposure to contaminants. Water collections were also conducted at two reference sites upstream of AOC contaminant point sources on Lake Huron at Wood Road (43.017817°, -82.018557°) and Port Franks (43.230722°, -81.903167°). Water and sediment collections in 2010 were conducted at the Port Franks reference site only.

Fertilized eggs used in both studies were collected in the spring as fresh egg masses from Beverly Swamp in Flamborough Township, Ontario (43.366800°, -80.116356°) in 2008 and from Valens Conservation Area (43.386211°, -80.131519°) in 2010. Both sites have healthy populations of frogs and eggs are readily available. These sites are ideal since there is minimal contaminant input from agricultural, industrial, or municipal runoff or effluent sources. Collections of eggs from these sites have been used for similar exposure studies done by ECCC at other Great Lakes AOCs.

For both exposure studies, eggs were raised in one litre hexane-cleaned glass jars containing 350 ml of fresh water collected from each of the study sites. Every other day, ten litres of water were collected from each of the study sites, placed into chemically clean four litre 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 reported in sediment collected from these locations in 2007–2009 (Drouillard 2010). Sediment was collected using a mini-ponar and placed in jars and water collections were performed 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 28–32 eggs per replicate were prepared for each study site for each experiment. For each replicate, eggs were checked and the water changed every 24 hours. Exposure studies were conducted at room temperature.

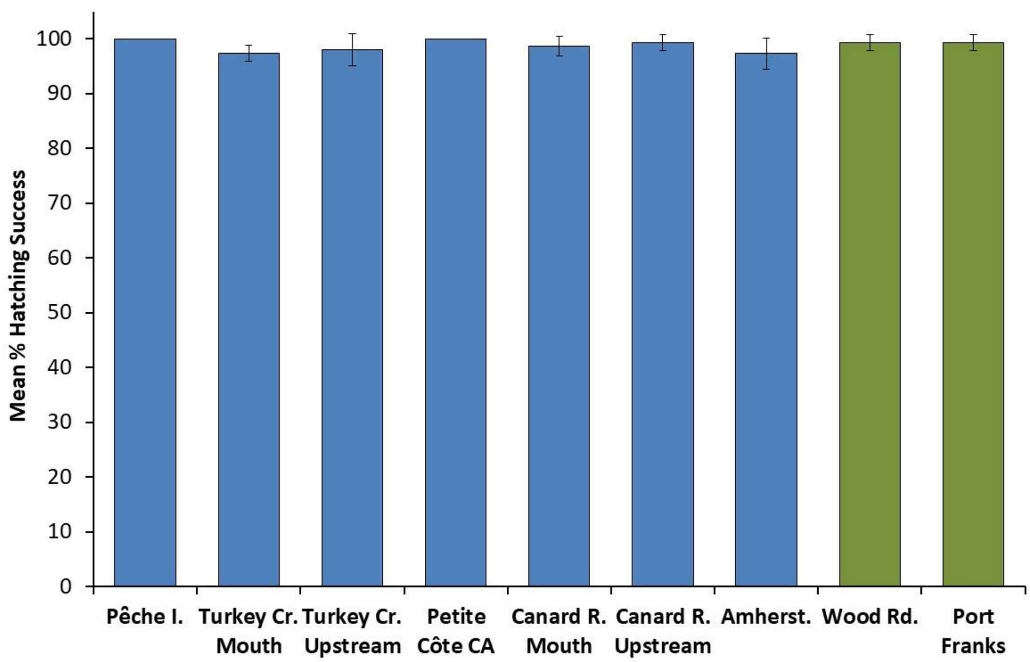
For both exposure studies, eggs were raised through hatching until they developed into free swimming tadpoles, Gosner stage 25, after approximately seven days. Tadpoles were then counted, euthanized using an overdose of MS-222 and preserved in 10% buffered formalin. Hatching success were calculated for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

Figure 1. Study locations in the Detroit River AOC where water (yellow circles) and water and sediment (red circles) samples were collected for the embryonic exposure studies conducted in 2008 and 2010, respectively (modified from Hughes *et al.* 2014).

Hatching success was high for eggs raised in water from seven Detroit River AOC locations in 2008 with mean hatching success ranging from 97.3% at Amherstburg to 100.0% at Pêche Island and Petite Côte Conservation Area (**Figure 2a**). No significant difference in hatching success was found among study sites including the two reference sites. Similarly, hatching success was also good in eggs exposed to sediment and water collected from five Detroit River AOC locations and the Port Franks reference site in 2010 with means ranging from 84.9% at Grosse Island Downstream to 89.0% at Trenton Channel (**Figure 2b**). No significant difference in hatching success was found among study sites.

Figure 2a. Mean hatching success (SD, as a percentage) of northern leopard frog eggs in raised in water in 2008 (a) and water and sediment in 2010 (b) collected from several Detroit River AOC locations (shown as blue bars) and upstream references sites on Lake Huron (Wood Road and Port Franks, shown as green bars). Five replicates of 28–32 eggs were tested per site (modified from Hughes *et al.* 2014).

a)



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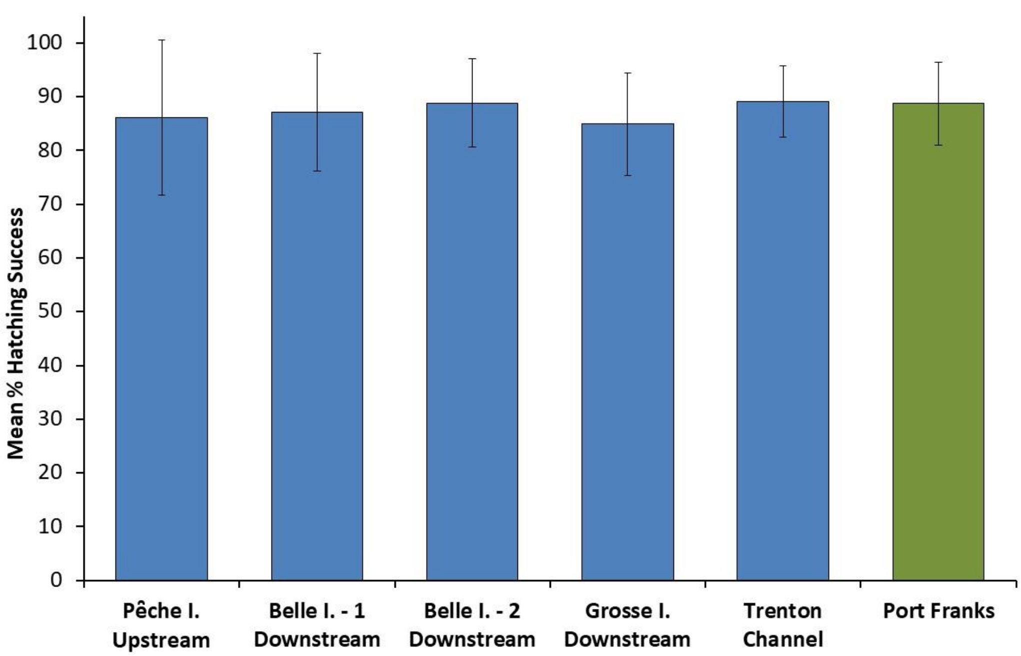
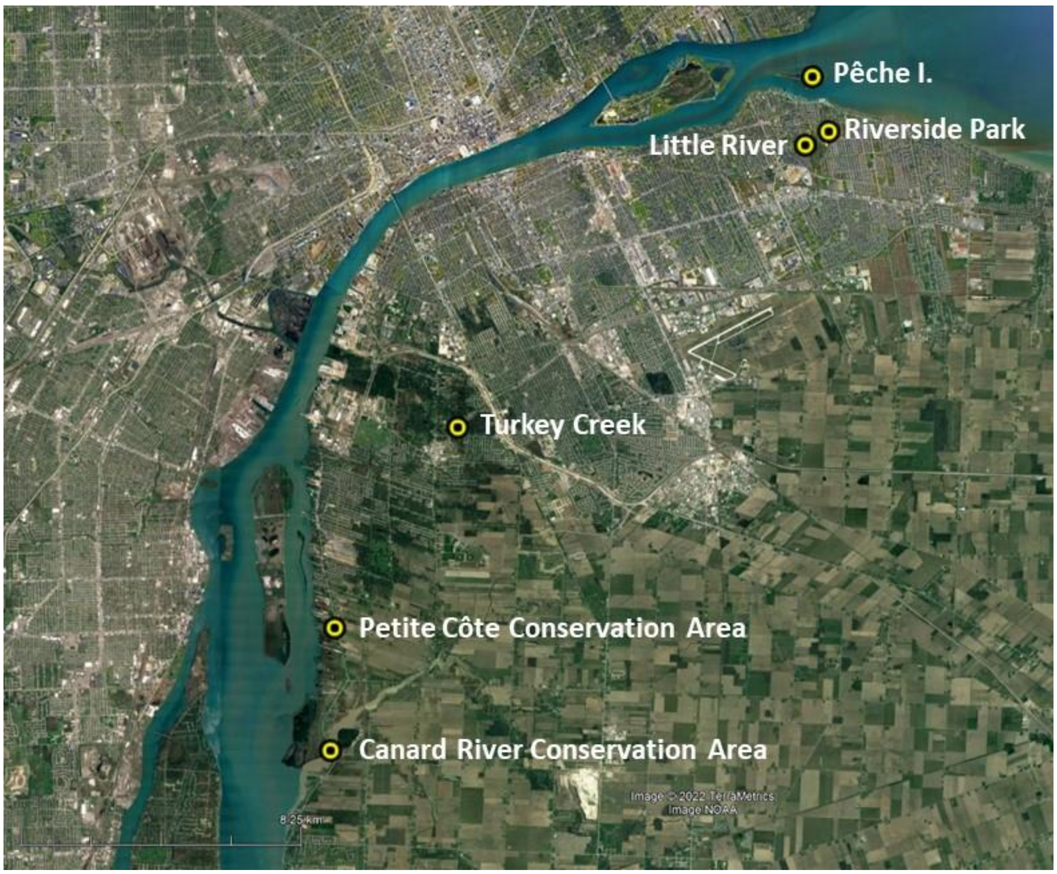


Contaminants in Northern Leopard Frogs

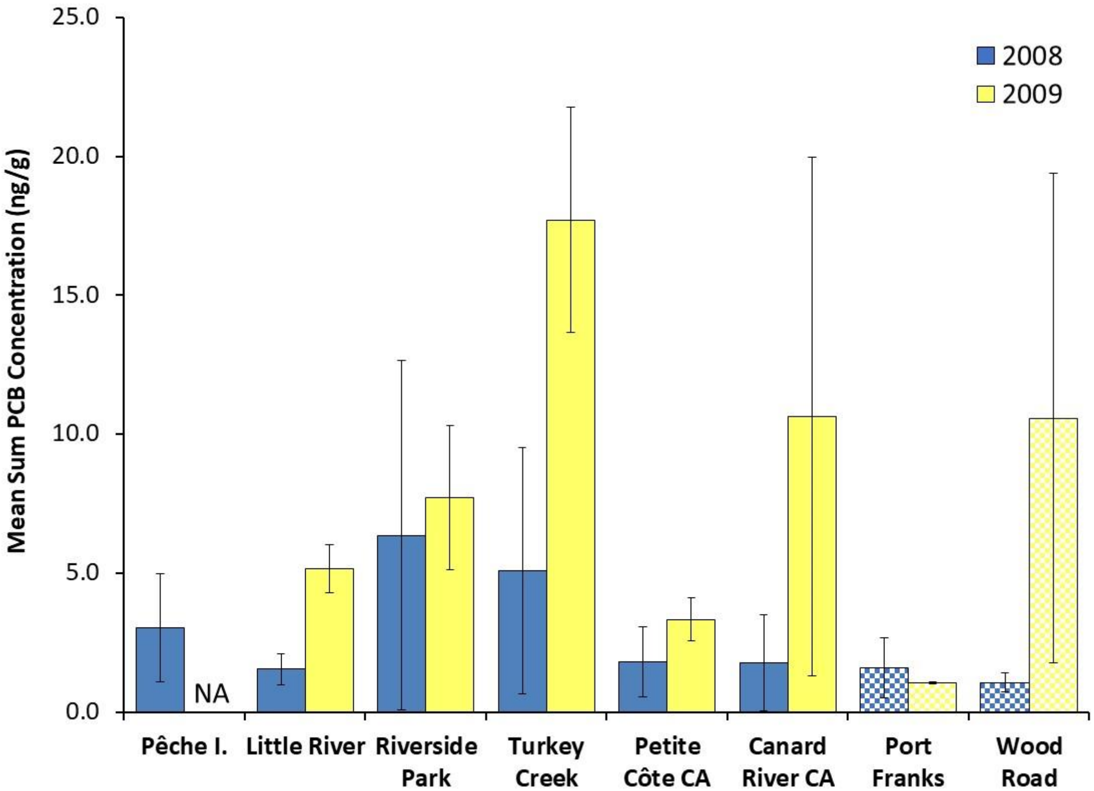
Frogs were collected in September of 2008 and 2009 from six Detroit River AOC locations and the two reference sites on Lake Huron (**Figure 3**). Whole bodies of frogs (minus gonads and spleens) were analyzed as individual frogs with 3–10 frogs analyzed per site (2008) or as pools consisting of 2–5 same sex frogs with three or four pools per site (2009). PCBs were quantified in frogs but mercury was not assessed.

Figure 3. Study locations in the Detroit River AOC where northern leopard frogs were collected to assess contaminant body burdens in 2008 and 2009 (modified from Hughes *et al.* 2014).



Mean sum PCB concentrations in frogs from six AOC locations ranged widely from 1.5 ng/g in frogs from Little River in 2008 to 17.7 ng/g in frogs from Turkey Creek in 2009 (**Figure 3**). Mean sum PCB concentrations were not consistently higher in frogs from AOC locations compared to the reference sites. PCB concentrations also varied widely between years at study sites. One frog from Petite Côte CA in 2008 had an extraordinarily high sum PCB concentration (102 ng/g) that was nearly twenty-five times higher than those in other frogs from that location; this outlier is not included in the mean shown in **Figure 3**. Nonetheless, PCB body burdens in this frog and all others from the AOC were below concentrations associated with toxic effects in amphibians. PCBs in frogs from the AOC were at least two orders of magnitude lower than levels associated with increased mortality in wood frog (*R. 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).

Figure 3. Mean concentrations (SD) of sum PCBs in northern leopard frogs collected from six Detroit River AOC locations (solid bars) and two upstream reference sites at Port Franks and Wood Road (hatched bars) in 2008 and 2009. One frog from Petite Côte Conservation Area with the maximum concentration in this study (102 ng/g) is not included here. NA indicates that data are not available.



Relatively higher sum PCB concentrations reported in frogs from Turkey Creek in 2009 supported the results of earlier trackdown monitoring activities in the Turkey Creek watershed that identified a localized area of PCB contamination at the Grand Marais Drain (Benoit and Burniston 2014). While remediation activities were completed in this area in 2008, relatively high PCBs in 2009 frogs may have been associated with disturbance at this site as a result of these activities. To fully assess the bioavailability of PCBs following remediation, additional monitoring of biota was required downstream along the Creek and at the creek mouth. Collections of snapping turtle eggs conducted from 2014–2016 were used to assess potential temporal changes in PCB exposure for biota on Turkey Creek as a non-lethal form of assessment (see below).

4. Black-crowned Night-Herons

Reproductive Success

Four species of colonial waterbirds have historically nested on the Canadian side of the Detroit River (Environment Canada 2010). In 2007, a new colonial waterbird species, the black-crowned night-heron, was reporting nesting on Turkey Island and, over the next few years, this became an established breeding site for night-herons.

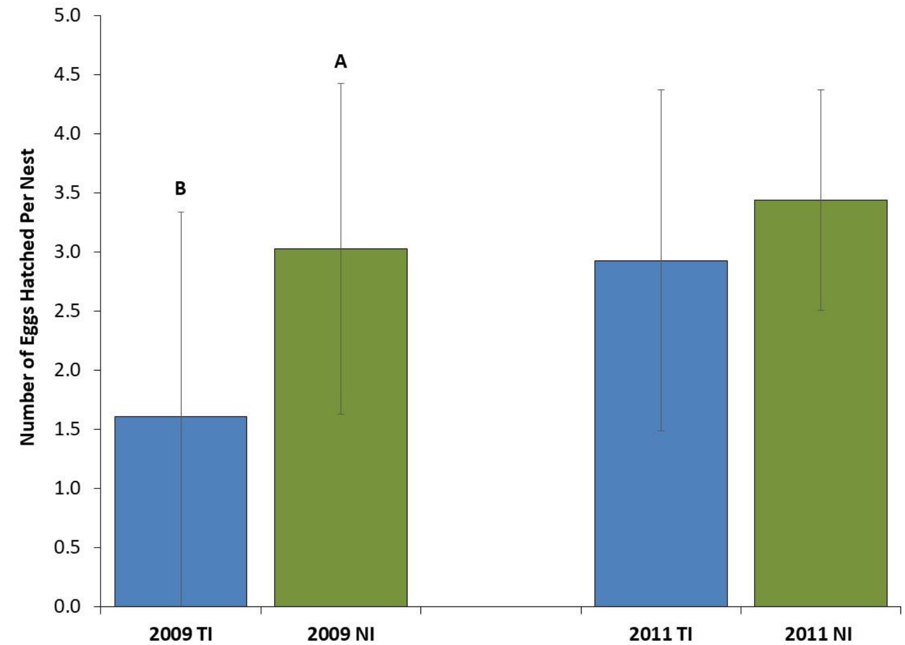
In 2009 and 2011, reproduction of night-herons was assessed by regular weekly visits to the Turkey Island colony (42.187°, -83.113°) in the Detroit River AOC from late April to mid-June. Similar visits were conducted at an upstream reference colony on Nottawasaga Island (44.538°, -80.258°) on Georgian Bay. During these visits, contents of each nest (i.e., the number of eggs or chicks present) and measurements of eggs and nestlings were recorded. Numbers of active nests monitored ranged from 28–34 nests at the two colonies in the two years. Various parameters of reproductive success were assessed that included hatching success as the number of eggs hatched per active nest and productivity as the number of chicks to reach 13 days of age per active nest. While night-heron chicks fledge at 15 days of age, chicks can leave the nest after 13 days of age and potentially hide in the surrounding dense tall stands of phragmites found at the AOC colony. To reduce the possibility of chicks escaping detection, this age served as a proxy for assessing fledging success.

Hatching success of night-herons at the AOC colony varied between the two study years (**Figure 4a**). In 2009, mean hatching success was significantly lower at the Turkey Island AOC colony (1.6 eggs hatched per nest) compared to the Nottawasaga Island reference colony (3.0 eggs hatched per nest). Further investigation of the fate of eggs in nests at the AOC colony in 2009 suggested that reduced hatching success was largely due to egg loss, i.e., eggs that disappeared or were destroyed, during the incubation period. Based on observations and conditions reported, predation and adverse weather events may have contributed to increased egg mortality at the AOC colony. Human disturbance associated with boat traffic can also influence adult incubation of eggs. Hatching success was relatively higher at both colonies in 2011, when approximately three eggs hatched per nest, compared to 2009 and no significant difference in hatching success was found between the two colonies. Clutch sizes were equal to four eggs per nest on average and clutch volumes (measured as total egg volume of four-egg clutches) were similar between the two colonies in the two study years. This suggests that food availability was not limited for birds at the AOC colony compared to the reference colony.

In 2009, productivity was also significantly lower at the AOC colony (as a mean, 1.1 chicks per nest) compared to the reference colony (2.2 chicks per nest; **Figure 4b**). In 2011, productivity was relatively higher at both the AOC colony (2.0 chicks per nest) and the reference colony (2.8 chicks per nest). Estimates of night-heron productivity at the AOC colony in 2011 and at the reference colony in both years were within the range of productivity estimates reported in several studies considered to be typical for a stable population (1.5–2.7 chicks per nest; refer to report for further details). In 2009, night-heron productivity was below this range of estimates. As reported above, low productivity in 2009 at the AOC colony was attributed to egg loss during the incubation period when only 43% of nests hatched at least one chick.

Figure 4. Mean number (SD) of black-crowned night-heron eggs hatched per nest (a) and productivity as the number of nestlings fledged per nest (b) at the Turkey Island (TI) colony in the Detroit River AOC and Nottawasaga Island (NI), the upstream reference colony, on Georgian Bay in 2009 and 2011. Different uppercase letters indicate a significant difference between colonies within a given study year.

a)



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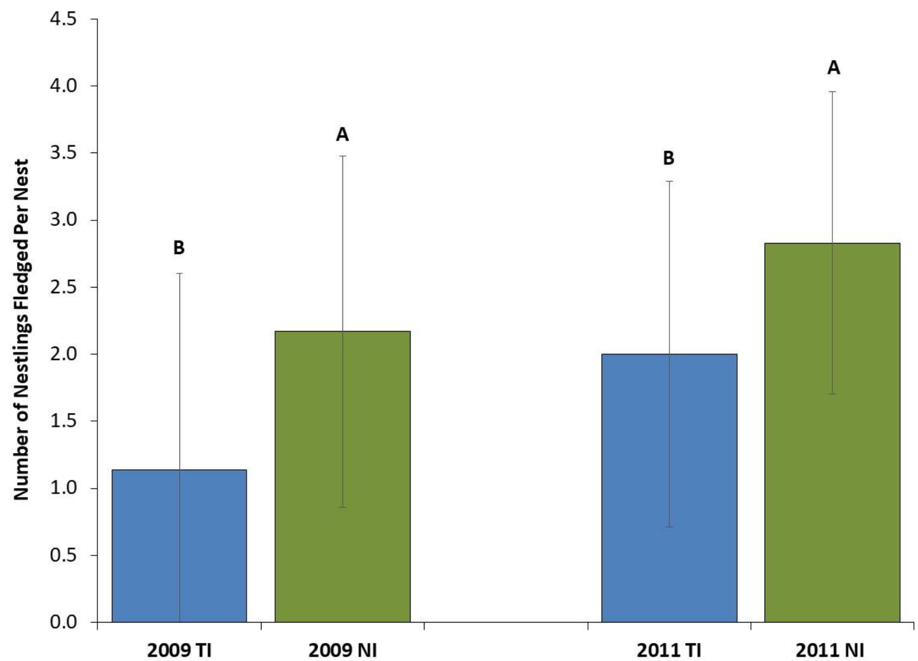


Contaminants in Night-Heron Eggs and Nestling Liver

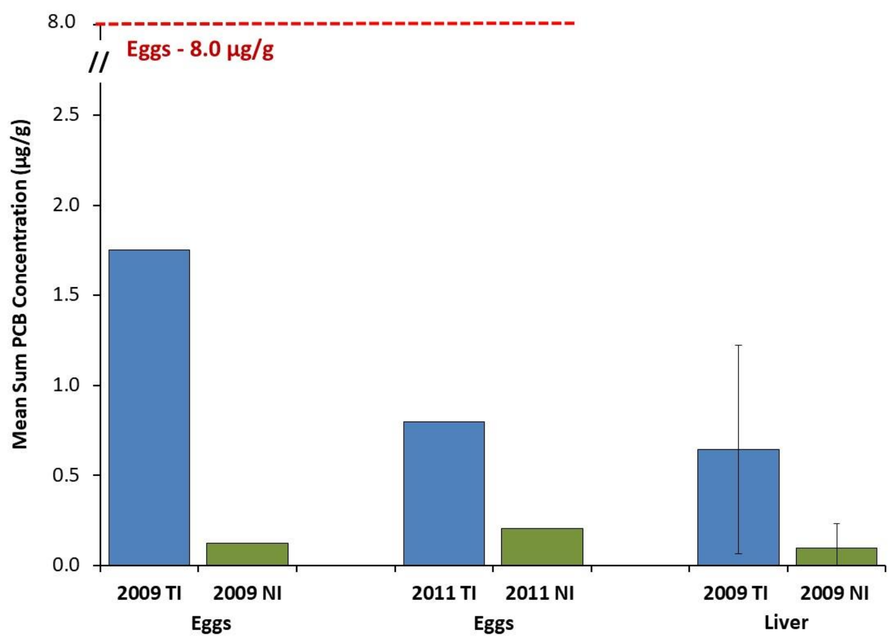
During the first visit to each colony in late April in 2009 and 2011, one fresh egg was collected from each of 10–12 individual 3- or 4-egg clutches from nests for contaminant analysis; these nests were not part of those monitored for reproductive success. Individual eggs were analyzed for mercury and as a single pool of eggs for organochlorines including PCBs. Night-heron are thought to be “income” breeders which means that they use resources acquired during the period of reproductive activity. However, it is possible that some maternal allocation of resources to eggs could have come from endogenous reserves (and thus lipophilic contaminants) acquired away from the breeding area. Given this, livers of six large pre-fledged night-heron nestlings were also analyzed individually in 2009 to provide an indication of local exposure to contaminants as birds forage and feed their young. Sum PCB concentrations are based on the sum of 34 or 62 individual or co-eluting PCB congeners in 2009 and 2011, respectively.

Sum PCB concentrations ranged from 0.13 µg/g in eggs from the Nottawasaga Island reference colony in 2009 to 1.75 µg/g in eggs from the Turkey Island AOC colony in 2009 (all single pooled samples; **Figure 5a**). Sum PCB concentrations in nestling liver in 2009 were relatively lower compared to eggs with mean concentrations of 0.65 µg/g and 0.10 µg/g at the AOC and reference colonies, respectively. Sum PCB concentrations in all egg samples were well below the 8–25 µg/g range that is associated with decreased hatching success in terns and cormorants (Hoffman *et al.* 1996). Sum PCB concentrations as high as 7–8 µg/g in eggs had no apparent impairment of night-heron egg hatching and chick survival (Custer *et al.* 1983). Concentrations were also at least four times higher in the two tissues in night-heron from the AOC colony compared to the reference colony in the two study years. While there is evidence of increased exposure to PCBs for night-heron foraging in the AOC, concentrations were likely not sufficiently elevated to adversely impact the breeding success of this species in the AOC.

Mean mercury concentrations in eggs from the two study colonies ranged from 0.21 µg/g at Nottawasaga Island reference colony in 2009 to 0.28 µg/g at Turkey Island AOC colony in 2011 and were not significantly different between colonies in the two study years (**Figure 5b**). Mercury concentrations in eggs of night-heron were generally well below the threshold concentration of 0.6 µg/g ww in eggs set to be *protective* against impairment of reproduction for 95% of non-marine avian species (Shore *et al.* 2011). This means that at egg mercury concentrations of up to 0.6 µg/g ww, no adverse impact on reproduction would be expected for 95% of species assessed. In this study, 93% of eggs analyzed (40/43 samples) were below this threshold concentration while three eggs exceeded this threshold concentration: one egg from Nottawasaga Island in 2009 (0.88 µg/g) and two eggs from Turkey Island in 2011 (0.64 µg/g and 1.01 µg/g). Henny *et al.* (2002) reported no adverse effects on reproduction for night-heron eggs containing 0.8 µg/g of total mercury, a concentration that was subsequently used to evaluate mercury-associated risk in several night-heron studies (e.g., Henny *et al.* 2007; Hill *et al.* 2008). Conestoga-Rovers & Associates (2012) assessed a range of relatively lower mercury toxicity thresholds for heron species in the literature against the 2009 mercury data and concluded that although mercury-related impacts to night-heron embryos cannot be ruled out, they are unlikely. A similar conclusion can be reached for 2011 eggs with few mercury concentration exceedances and good hatchability of eggs in the AOC. Based on several lines of evidence, it is unlikely that hatchability and development of night-heron embryos would be negatively impacted in eggs with the highest mercury concentrations.

Figure 5. Mean concentrations (SD) of sum PCBs (a) and total mercury (b) in black-crowned night-heron eggs and nestling liver from the Turkey Island (TI) colony in the Detroit River AOC and Nottawasaga Island (NI), the upstream reference colony, on Georgian Bay in 2009 and 2011. Sum PCB concentrations for eggs are based on analysis of a single pooled sample comprised of 10–12 eggs. Threshold concentrations (dotted red lines) indicate concentrations in eggs and liver (where available) above which a potential adverse impact on reproduction might be expected.

a)



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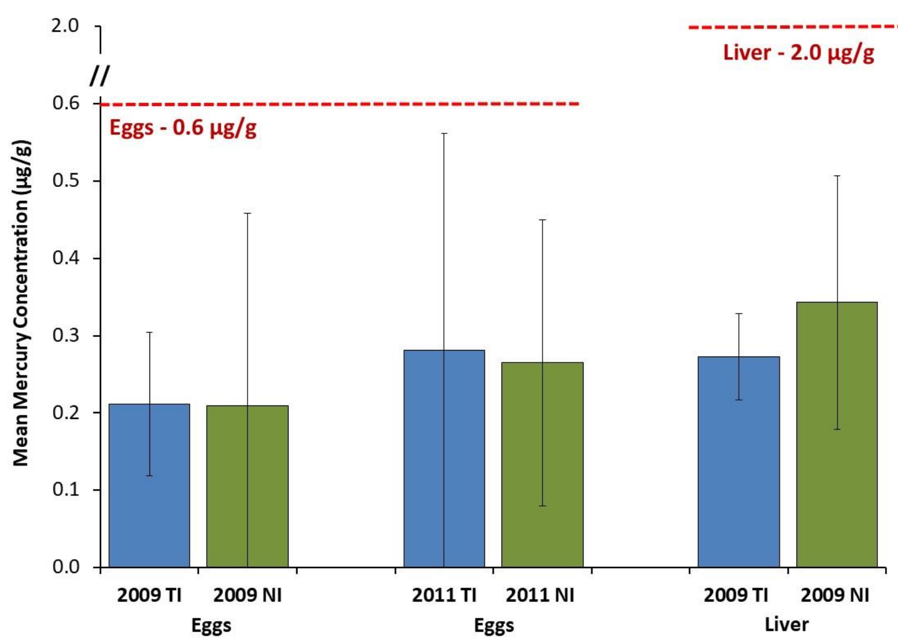


Hepatic (liver) mercury concentrations in nestlings were statistically similar between the two colonies with a maximum liver concentration of 0.66 µg/g in a nestling from Nottawasaga Island in 2009 (**Figure 5b**). Compared to sum PCBs, mercury concentrations were more similar between eggs and nesting livers at both study sites. Mercury concentrations in all liver samples were well below that the threshold concentration of 2.0 µg/g ww set to be *protective* against adverse reproductive effects for 95% of non-marine avian species (Shore *et al.* 2011).

Reduced reproductive success of night-heron at the AOC colony in 2009 was likely due to stressors such as predation, weather, and disturbance and not due to elevated concentrations of legacy contaminants historically associated with the AOC. Relatively higher hatching success and productivity reported at the AOC colony in 2011 compared to 2009 suggests the intensity of these stressors may vary from year to year. Black-crowned night-heron no longer nest on the Canadian side of the Detroit River with the last recorded nests (six) reported in 2012.

5. Snapping Turtles

Hatching Success

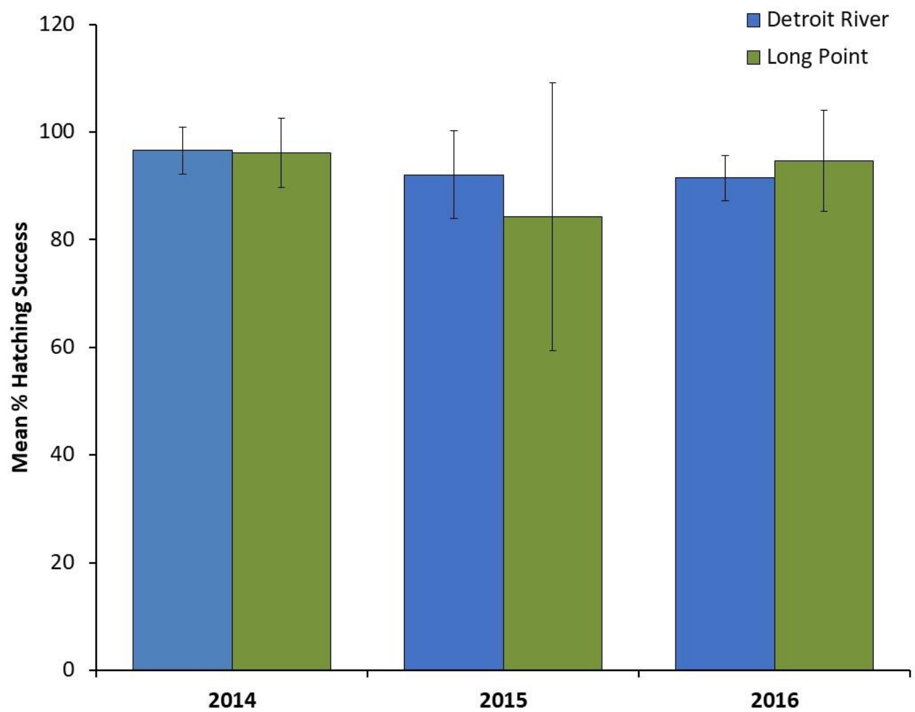
Snapping turtle eggs were collected from several sites in the Detroit River AOC in June 2014, 2015, and 2016 (**Figure 6**). Coastal wetlands at the outflows of Turkey Creek and Canard River as well as sites along the creeks and drains that flow into these creeks were targeted as these areas support suitable nesting habitat for turtles. Ten clutches of eggs were targeted in each year but this was not always achievable at Turkey Creek and Canard River where much of the land is privately owned and access to nesting sites was limited. With considerable effort, five clutches were collected in 2014 and 2016 and twelve clutches were collected in 2015 in the AOC. One additional clutch was collected in Ojibway Park close to the Ojibway Nature Centre in 2014. This area is largely protected, remote from point sources, and no tributary flows into the Detroit River from this site. As such, it is not considered part of the AOC for this assessment but is shown here for comparison purposes as a relatively clean site. Ten clutches of eggs were collected from a reference site at Long Point on the shore of Lake Erie (42.577080°, -80.446783°) in each of the three study years.

Entire clutches of eggs were collected within 48 hours of oviposition, placed in plastic containers containing moistened vermiculite, and artificially incubated at approximately 24°C in a laboratory at the Canada Centre for Inland Waters (CCIW) in Burlington, Ontario until the time of hatching. Hatching success were calculated for each clutch as the number of eggs hatched relative to the number of eggs incubated. 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 6. Study locations where clutches of snapping turtle eggs were collected in the Detroit River AOC to assess hatching success and contaminant burdens from 2014–2016. Note that symbols may represent the location of multiple clutches. The single clutch collected in Ojibway Park in 2014 is not considered part of the AOC since this site is inland and not influenced by point sources on the River (modified from Hughes *et al.* 2018).

Hatching success of snapping turtle eggs collected from the Detroit River AOC in the three study years ranged, as means, from 91.5% in 2016 to 96.6% in 2014 (**Figure 7**). Hatching success was not significantly different clutches between AOC sites and Long Point reference site in each of the three study years. All 18 eggs hatched (100%) in the single clutch collected in 2014 from Ojibway Park, an inland site that has no hydrological connection to the Detroit River.

Figure 7. Mean hatching success (SD, as a percentage) in clutches of artificially-incubated snapping turtle eggs collected from sites in the Detroit River AOC and the reference site at Long Point on Lake Erie from 2014–2016.



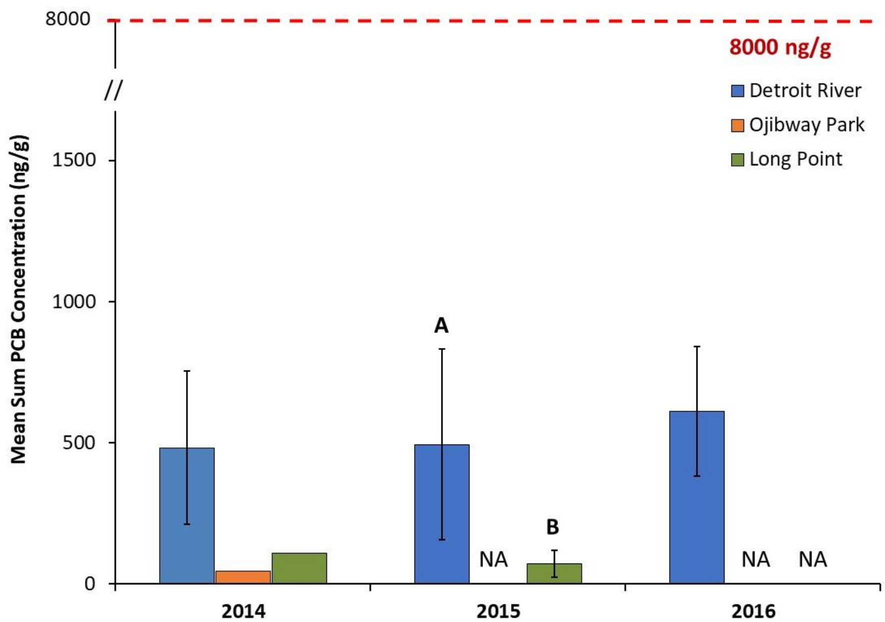
Contaminants in Turtle Eggs

In 2014 and 2015, approximately five eggs per clutch were randomly selected and sent for contaminant analysis at the time of egg collection. In 2016 however, whole newly-hatched turtles that had emerged from incubated eggs were chemically analyzed for contaminants (five per clutch). Differences in PCB burdens between freshly laid eggs and newly hatched turtles are not large, i.e., less than 15%, and as such are generally comparable; further details regarding comparability of concentrations are provided in the report. Eggs from the Long Point reference site were analyzed as a single pooled sample in 2014 and as individual clutches (10) in 2015; hatchlings from Long Point were not assessed for contaminants in 2016. Concentrations are reported on a wet weight basis for PCBs and as dry weights for total mercury.

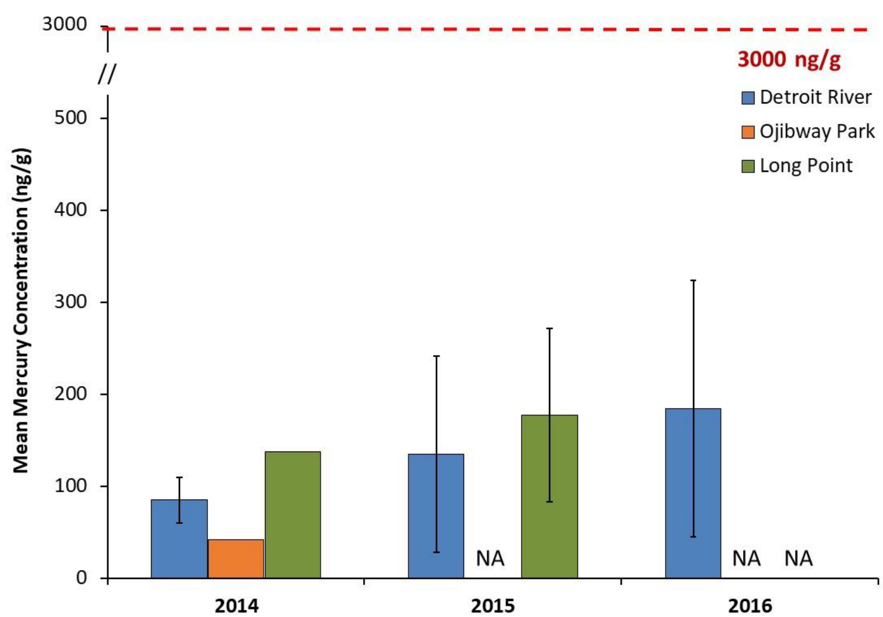
Sum PCB concentrations were higher in snapping turtle eggs from sites in the Detroit River AOC compared to the Long Point reference site in 2014 and 2015 (**Figure 8a**). This difference was significant in 2015 when multiple clutches of Long Point eggs were chemically analyzed and a comparison could be assessed statistically. Mean sum PCB concentrations were highest in hatchling turtles in the AOC in 2016 compared to eggs collected in earlier years when assessed both as wet weight concentrations and lipid-weight concentrations (data not shown). When site data were combined across years, mean sum PCB concentrations were highest in eggs and hatchlings from Turkey Creek compared to those from Canard River and Little River. As a site hydrologically separated from the Great Lakes and upstream point source influences, the single clutch from Ojibway Park in 2014 had the lowest PCB concentrations of all study sites.

Figure 8. Mean concentrations (SD) of sum PCBs (a) and total mercury (b) in snapping turtle eggs from sites in the Detroit River AOC and the reference site at Long Point on Lake Erie from 2014 and 2015. Mean (SD) concentrations are shown for newly hatched turtles in clutches from the AOC in 2016. The single clutch collected in Ojibway Park in 2014 is not considered part of the AOC. Threshold concentrations (dotted red lines) indicate concentrations in eggs above which a potential adverse impact on reproduction might be expected. Different uppercase letters indicate a significant difference between the AOC and reference site in 2015. Concentrations are in ng/g as wet weights for PCBs and as dry weights for total mercury. NA indicates that data are not available.

a)



b)



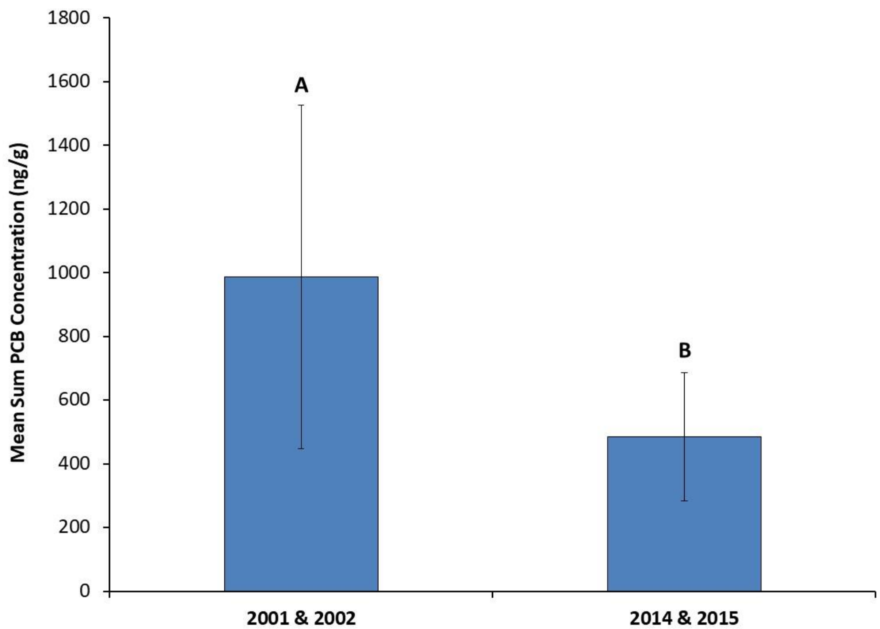
In contrast to birds, PCB effect-level threshold concentrations above which adverse effects on survival and reproduction might be expected have not been proposed for reptiles. Using egg PCB thresholds identified for birds as a surrogate for turtles (since both are amniotes and lay eggs), a broad literature review of PCB effects in birds recommended that egg PCB concentrations in the range of 8000 to 25000 ng/g were associated with decreased hatching success for terns and cormorants (Hoffman *et al*. 1996). In this study, sum PCB concentrations in turtle eggs from the AOC were well below the lower threshold with a maximum concentration of 1300 ng/g. Sum PCB concentrations in AOC eggs were also likely below those expected to induce expression of a gene in the liver that is involved in the breakdown and removal of PCBs from the body. Colson *et al.* (2021) reported a significant increase in expression of this gene in a dosing study of snapping turtles at a liver sum PCB concentration of 3.54 µg/g (wet weight). Based on the maximum egg PCB concentration in this study and using a ratio to estimate a corresponding liver concentration (Pagano *et al.* 1999; de Solla and Fernie 2004), the estimated liver sum PCB concentration (2.31 µg/g) in this turtle is well below the concentration associated with induction of this gene.

Mercury concentrations in eggs collected from sites in the Detroit River AOC in 2014 and 2015 were largely similar to, or lower than, concentrations in eggs from Long Point reference site (**Figure 8b**). In 2015, no significant difference was found in mean mercury concentrations between AOC and reference site eggs. Similar to PCBs, few studies have examined mercury thresholds associated with the adverse reproductive effects in reptiles. One study reported that an average mercury concentration of 3000 ng/g dry weight (dw) in snapping turtle eggs was associated with a 12% reduction in hatching success compared to reference sites (Hopkins *et al.* 2013). The maximum mercury concentration in eggs in this study was 401 ng/g dw (or 84.5 ng/g wet weight) in a clutch from Canard River in 2015 and was well below this effect-level concentration. High hatching success reported at the AOC site in the lab incubation study using eggs from these clutches support this conclusion. In addition, using the egg mercury threshold for birds as a surrogate for turtles, mercury concentrations in eggs of turtles were well below the threshold concentration of 600 ng/g ww in bird eggs set to be *protective* against adverse reproductive effects for 95% of non-marine avian species (Shore *et al.* 2011). Overall, concentrations of PCBs and mercury were not sufficiently elevated to adversely impact the reproductive success of snapping turtles in the AOC.

Historic PCB contamination was identified in the upper reaches of Turkey Creek in sediment, soil, water and fish following trackdown monitoring activities conducted from 2001–2006 (Benoit and Burniston 2014). Following this, remediation activities that involved removal of approximately 975 m^3 of PCB-contaminated sediment from the Grand Marais Drain – that is connected to Turkey Creek – was completed in 2008. To assess the effectiveness of these activities at this site using turtles as biomonitors, contaminant burdens in eggs were compared between egg collections on Turkey Creek in 2001 and 2002 and those in the current study. A significant decrease in sum PCB concentrations was found between the two studies in which mean sum PCB concentrations decreased by 51% in clutches from 2014 and 2015 compared to 2001 and 2002 (**Figure 9a**). For mercury, no change in concentrations were found between egg collections in 2002 and 2014 and 2015 on Turkey Creek (**Figure 9b**). While this remediation site is several kilometres upstream from clutch collection sites, it is likely that remediation of this site has been important in decreasing PCB exposure to biota further downstream.

Figure 9. Mean concentrations (SD) of sum PCBs (a) and total mercury (b) in eggs of snapping turtles from collection sites on Turkey Creek that were downstream of the Grand Marais Drain remediation site in 2001 and/or 2002 and 2014 and 2015 (current study). Means are based on collections of 7 or 8 clutches. Different uppercase letters indicate a significant difference between the early study and the current study. Sum PCBs are based on sum concentrations of 25 common PCB congeners analyzed over these years. Concentrations are in ng/g as wet weights for PCBs and as dry weights for total mercury.

a)



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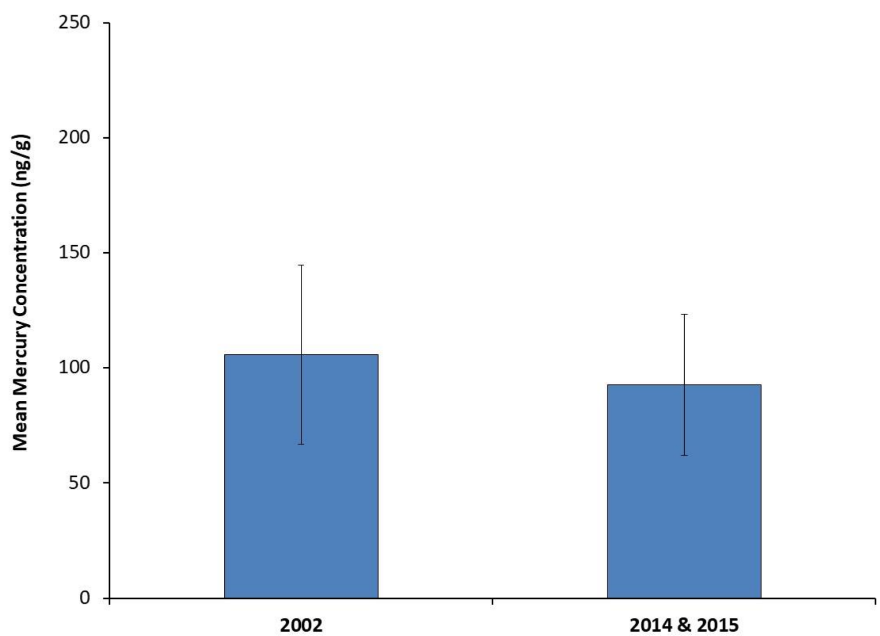


6. Double-crested Cormorants & Herring Gulls

Embryonic Viability

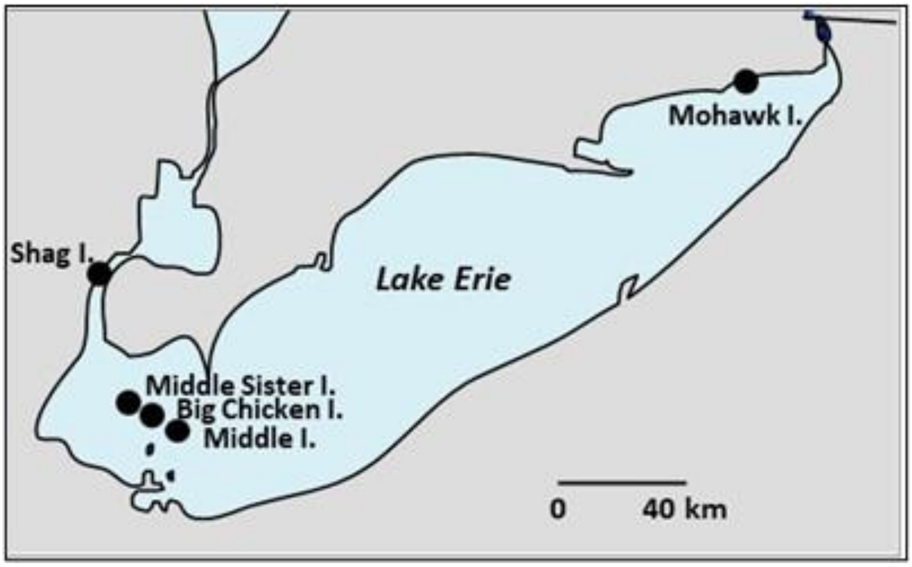
Large scale changes in populations of breeding colonial waterbirds on the Canadian side of the Detroit River have been evident over the last two decades. In 2007, over 29,000 colonial waterbird nests (=breeding pairs), comprised largely (96%) of ring-billed gull (*Larus delawarensis*) nests, were recorded on the Canadian side of the River. Since then, numbers of breeding pairs of colonial waterbirds have declined precipitously and by 2013, there was no evidence of colonial waterbirds nesting on this side of the River. Herring gulls were last reported nesting in 2010. Efforts to continue ongoing annual collections of herring gull eggs for contaminants monitoring as part of the Great Lakes Herring Gull Monitoring Program and conduct assessments of nesting gulls on the Canadian side of the River were no longer possible.

In 2012, a small colony of double-crested cormorants was reported nesting near the middle of the River and close to the Canada-United States international border. This colony was located on an unnamed island west of Fighting Island and approximately 170 metres off the northern tip of Grassy Island in Michigan. For the purpose of reporting, this AOC site will be referred to as Shag Island (**Figure 10**). By 2015, the breeding colony of cormorants was of a sufficient size to begin an assessment of reproduction in a second colonial waterbird species following the completion of the black-crowned night-heron study on Turkey Island in 2011.

Double-crested cormorant eggs were collected from Shag Island from 2015–2019. The next closest colonial waterbird colony in Canada is at Middle Sister Island situated approximately 21 km downstream of the AOC boundary in western Lake Erie (**Figure 10**). Two reference colonies that were removed from the local sources of pollutants on the Detroit River were selected for comparison purposes: Black Rock (46.116216^{o}, -82.833935^{o}) in Lake Huron (collections in 2016) and Mohawk Island in eastern Lake Erie (collections in 2018 and 2019; **Figure 10**)

In early April of 2015, 2016, 2018 and 2019, freshly laid cormorant eggs (i.e., not incubated) were collected from nests at Shag Island, Middle Sister Island, and reference colonies for artificial incubation in the laboratory. Eggs were collected from nests containing a single egg (where possible, since some nests could not be easily accessed), transported to NWRC in insulated coolers with foam inserts, set in a humidity-controlled incubator at 37^{o}C, and turned every two hours. Just prior to the pipping stage of development (i.e., embryonic day 26–27), embryos were removed from their shells and euthanized by decapitation. Embryonic viability was determined as the number of viable embryos that survived to the designated embryonic day (i.e., just prior to pipping) divided by the total number of fertile eggs. Due to some logistical challenges in the 2015 pilot study year, viability could not be reliably assessed in eggs; however, these embryos were used for the assessment of contaminant burdens (see below). Viability was also not assessed in embryos from Middle Sister Island in 2018 due to a problem with the incubator. Numbers of eggs collected for artificial incubation from each colony ranged from 9–35 in the three study years.

Figure 10. Colony locations of double-crested cormorants where eggs were collected to assess embryonic viability and contaminant burdens from 2015–2019. Shag Island is Detroit River AOC colony, Middle Sister Island is the downstream near-AOC colony, and Mohawk Island and Black Rock (on Lake Huron, not shown) are the reference colonies. Annual collections of cormorant eggs have also been performed for contaminant analysis at Middle Island.

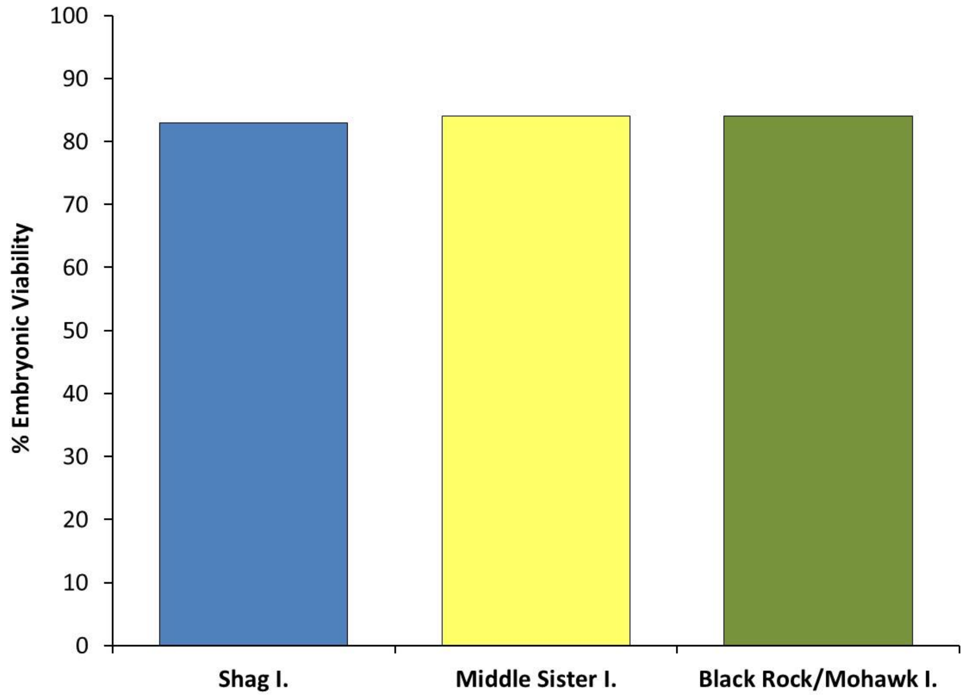


Embryonic viability was 83% in double-crested cormorant eggs collected from the Detroit River AOC colony at Shag Island in the three study years in 2016, 2018, and 2019 (data combined; **Figure 11**). Embryonic viability was very similar at the downstream near-AOC colony at Middle Sister Island (84%) in 2016 and 2019 (when this could be assessed) and at the two reference colonies at Black Rock and Mohawk Island (84%) in the three years. There was no evidence of reduced embryonic viability in cormorant eggs for birds foraging in the AOC.

Contaminants in Cormorant Embryos and Eggs

Whole embryo carcasses, egg contents (including yolk sac), and shell membranes were collected in chemically-cleaned glass jars, homogenized, and frozen until chemical analysis. Embryos were analyzed individually for PCBs at Shag Island in 2016 (four embryos) and as a single pooled sample consisting of three embryos in 2015 at Shag Island, Middle Sister Island, and Black Rock and in 2017 at Middle Sister Island and Mohawk Island. Mercury analysis was conducted for collections of 2016 and 2017 embryos only. As part of the Great Lakes Herring Gull Monitoring Program, annual collections of cormorant eggs for contaminant analysis have been performed at Shag Island, Middle Island (**Figure 10**, downstream of Middle Sister Island on Lake Erie), and Black Rock from 2015–2019 and results are included here for comparison purposes. Eggs were analyzed as single pools at each colony in each of the five study years.

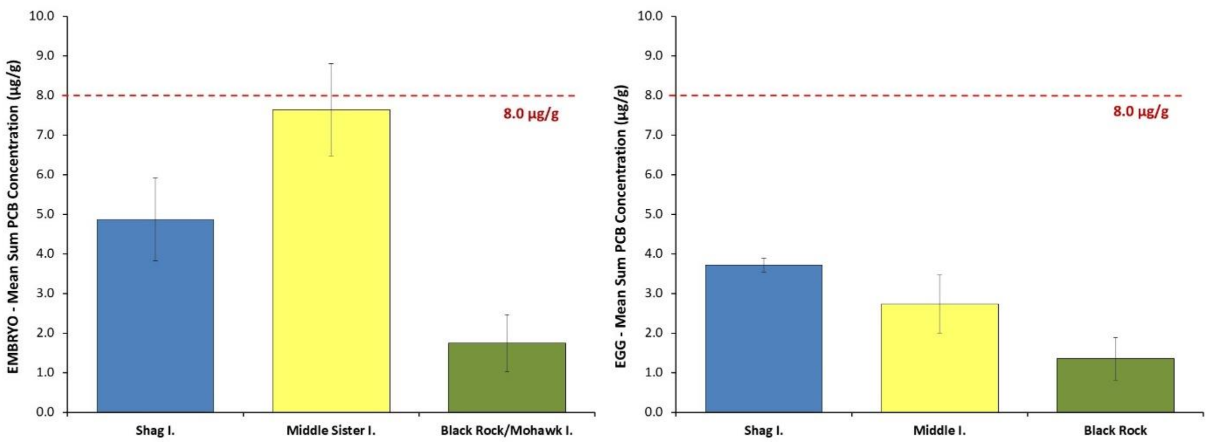
Figure 11. Embryonic viability in artificially incubated double-crested cormorant eggs collected from the Detroit River AOC colony (Shag Island), a downstream near-AOC colony (Middle Sister Island, Lake Erie) and reference colonies outside the AOC (Black Rock, Lake Huron and Mohawk Island, Lake Erie) in 2016, 2018, and 2019.



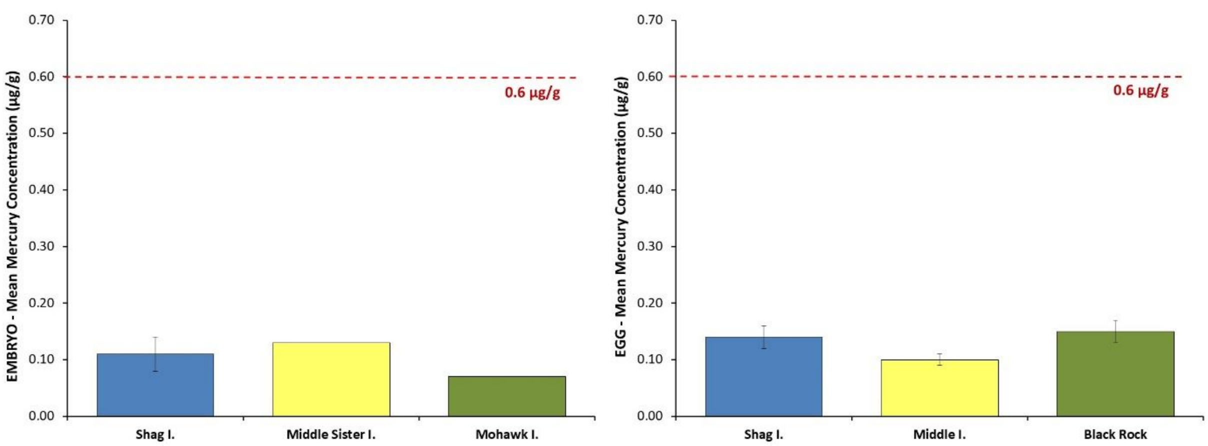
For all years combined, mean sum PCBs in embryos were highest at the downstream near-AOC colony at Middle Sister Island (7.6 µg/g) followed by the AOC colony at Shag Island (4.9 µg/g) and then the reference colony at Black Rock/Mohawk Island (1.8 µg/g) from 2015–2017 (**Figure 12a**). Sum PCB concentrations in embryos from Shag Island in 2015 (single pool) and 2016 (four individuals) ranged from 3.9 µg/g to 6.7 µg/g. A different spatial pattern was found for sum PCBs in cormorant eggs. Sum PCB concentrations in cormorant eggs were the highest at Shag Island (3.7 µg/g), followed by Middle Island (2.7 µg/g), and Black Rock (1.4 µg/g; **Figure 12a**). Several factors may contribute to these differences in spatial patterns for PCBs between embryos and eggs. Colonial waterbirds nesting at Middle Sister Island, due to its proximity to the AOC, may be more directly impacted by Detroit River contaminant sources than those from Middle Island which is further downstream. Cormorants nesting at Middle Sister Island are also within foraging distance of the Detroit River. Contaminant sources from other AOCs in western and eastern Lake Erie may also contribute to higher exposure in cormorants from Middle Sister Island compared to Middle Island. Sum PCB concentrations in cormorant embryos and eggs from the AOC colony were below the 8–25 µg/g range that has been associated with decreased hatching success for terns and cormorants (Hoffman *et al.* 1996).

Figure 12. Mean concentrations (SD) of sum PCBs (a) and total mercury (b) in double-crested cormorant embryos and eggs from Detroit River AOC colony (Shag Island), a downstream near-AOC colony (Middle Sister Island, Lake Erie) and reference colonies outside of the AOC (Black Rock and Mohawk Island). Means are based on collections from 2015–2017 for embryos and 2015–2019 for eggs. Threshold concentrations (dotted red lines) indicate concentrations in eggs above which a potential adverse impact on reproduction might be expected.

a) Sum PCBs



b) Mercury



Mercury concentrations in embryos from Shag Island and Middle Sister Island were very similar and higher than the embryos from the reference colony at Mohawk Island in eastern Lake Erie (**Figure 12b**). The maximum mercury concentration was found in an embryo from Shag Island (0.15 µg/g). In fresh eggs, mean mercury concentrations were highest in those from reference colony at Black Rock on Lake Huron and lowest at the downstream AOC colony at Middle Island (**Figure 12b)**. Mercury concentrations in AOC embryos and eggs were well below the predicted threshold of 0.6 µg/g in eggs set to be *protective* against adverse reproductive effects for 95% of non-marine avian species (Shore *et al.* 2011). Overall, concentrations of PCBs and mercury were not sufficiently elevated to adversely impact the reproductive success of cormorants in the AOC.

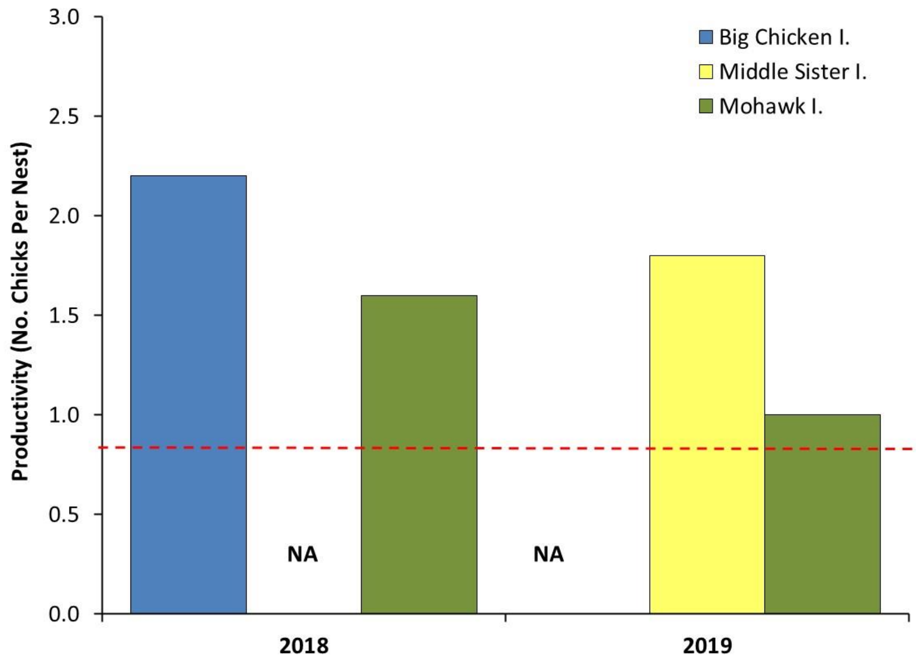
Herring Gull Productivity

Productivity could not be assessed in cormorants nesting at the AOC colony on Shag Island due to few accessible nests and fewer nests available for study with each passing year. The island is very small and consists of a few trees on a pile of rocks. While herring gulls have not nested on the Canadian side of the Detroit River AOC since 2010, they have nested for decades at two large well-established colonial waterbird colonies downstream in western Lake Erie. Following this, two surrogate AOC colonies on Middle Sister Island and Big Chicken Island were selected for assessments of gull productivity in wild populations. While beyond the boundary of the AOC, they are directly influenced by localized contaminant sources on the Detroit River due to their location downstream of the AOC. Historic spatial trends for PCBs in gull eggs in 1994 and 2002 support this. Sum PCB concentrations were at least 19% higher in gull eggs from Middle Sister Island compared to those from Fighting Island (within the AOC) in these two years (ECCC unpublished). A similar spatial pattern was found in cormorants in the current study with higher PCB burdens in embryos from Middle Sister Island compared to Shag Island (**Figure 12a**). This field assessment of numbers of chicks produced per breeding pair in the wild complements those assessing embryonic viability in bird eggs in the laboratory. Productivity was assessed at the two surrogate AOC colonies and at the reference colony on Mohawk Island in eastern Lake Erie in 2018 and 2019 (**Figure 10**). Unfortunately, storm events at Middle Sister Island in 2018 and at Big Chicken Island in 2019 destroyed enclosures and no productivity estimates could be made at these colonies in these years.

Visits to study colonies were conducted at two times during the breeding season: 1) egg laying (late April) and 2) when gull chicks were $\geq$ 21 days old (early-June) to assess reproduction and other health endpoints in chicks. During the first visit, a thorough nest count was conducted and contents were recorded at the colony. Individual nest enclosures (~1m in diameter and 16" high) were constructed around twelve 3-egg nests at each colony for monitoring of chicks. Egg measurements were recorded for thirty 3-egg nests at each colony. During the second visit when chicks in enclosed nests were >21 days old, productivity was calculated at the number of chicks divided by the number of enclosed nests.

Herring gull productivity, defined as the number of $\geq$ 21-day-old chicks produced per nest, was equal to 2.2 chicks per nest at Big Chicken Island in 2018 (N=9 enclosed nests) and 1.8 chicks per nest at Middle Sister Island in 2019 (N=8 nests; **Figure 13**). Productivity at the reference colony was slightly lower at 1.6 chicks per nest in 2018 and 1.0 chicks per nest in 2019 (N=7 enclosed nests in both years). Overall, productivity estimates at all colonies in the two study years were within, or exceeded, the range of productivity levels required to maintain a stable population (0.8–1.4 chicks per nest; Kadlec and Drury 1968). Total clutch volumes (measured as total egg volume of three-egg clutches) in eggs from the two surrogate AOC colonies were similar to the Mohawk Island reference colony in 2018 and 2019 (data not shown). This suggests that food availability at the time of egg laying was not limited for birds at these surrogate colonies compared to the reference colony.

Figure 13. Herring gull productivity, as the number of ≥ 21-day-old chicks produced per nest, at the two surrogate AOC colonies (Big Chicken Island and Middle Sister Island) and the reference colony (Mohawk Island) in 2018 and 2019. The red dashed line indicates the minimum productivity level of 0.8 chicks per nest associated with maintaining a stable herring gull population (range in levels=0.8–1.4 chicks per nest; Kadlec and Drury 1968). NA indicates that data are not available.



7. Tree Swallows

Reproductive Success

Twenty five nest boxes were erected along the shoreline of the Detroit River in November 2015 at each of the three primary study sites that were downstream of industrial and municipal discharges to the River. These sites were at Windsor Salt in Windsor, Riverdance Park in LaSalle, and White Sands Conservation Area (CA) on Boblo Island in Amherstburg (**Figure 14**). Two sites were upstream of Detroit River point sources at Sandpoint Beach Park and Shanfield Shores Park and combined these served as the upstream, albeit within the AOC, reference site (**Figure 14**). Importantly, this reference site also assesses the influence of upstream sources of pollutants on the St. Clair River (another AOC) and Lake St. Clair to the Detroit River. In general, tree swallows readily occuped nest boxes and reproductive success was monitored at sites annually from 2016–2019.

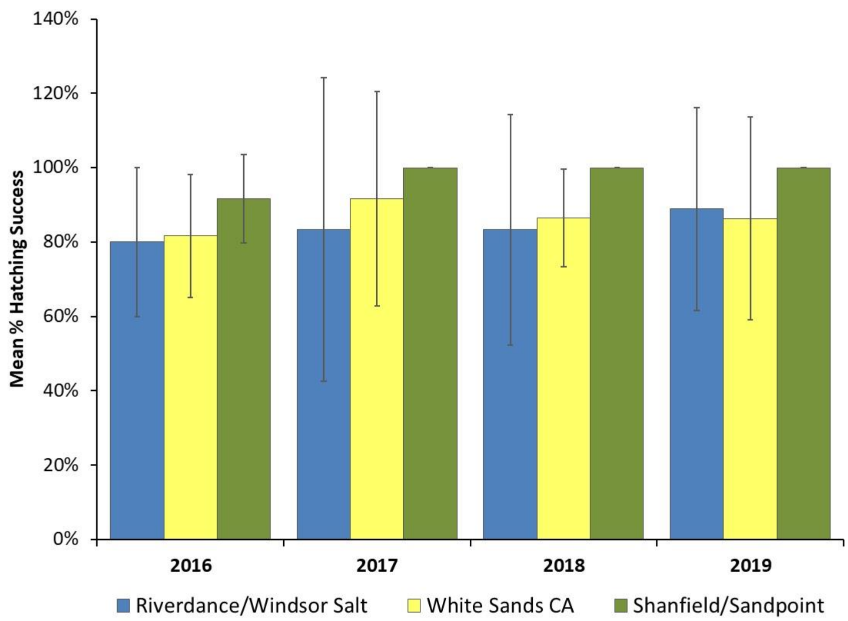
Nest boxes were visited beginning in late April or early May when tree swallows had returned to the area to begin nesting. In general, visits to nest boxes were conducted every 3–5 days early in the breeding cycle with timing coordinated to dates for egg hatching and age of nestlings at 16 days. Various parameters of reproductive success were assessed that included hatching success, as the number of eggs hatched divided by the total number of eggs in the clutch, and fledging success as the number of nestlings present in the nest at day 16 divided by the number of eggs that hatched; both parameters are expressed as percentages. While tree swallow chicks fledge at 23 days of age, if the chicks are disturbed after 16 days of age, there is an increased risk that they may be scared out of the boxes too early resulting in reduced survival; consequently, this age was used as a proxy for assessing fledging success. A final visit was conducted at day 23 to check for dead nestlings remaining in the box. Nest boxes were emptied and cleaned out in the fall each year.

Figure 14. Study locations of four tree swallow nesting sites monitored within the Detroit River AOC from 2016–2019. The upstream reference site is at Sandpoint Beach Park and Shanfield Shores Park (combined).

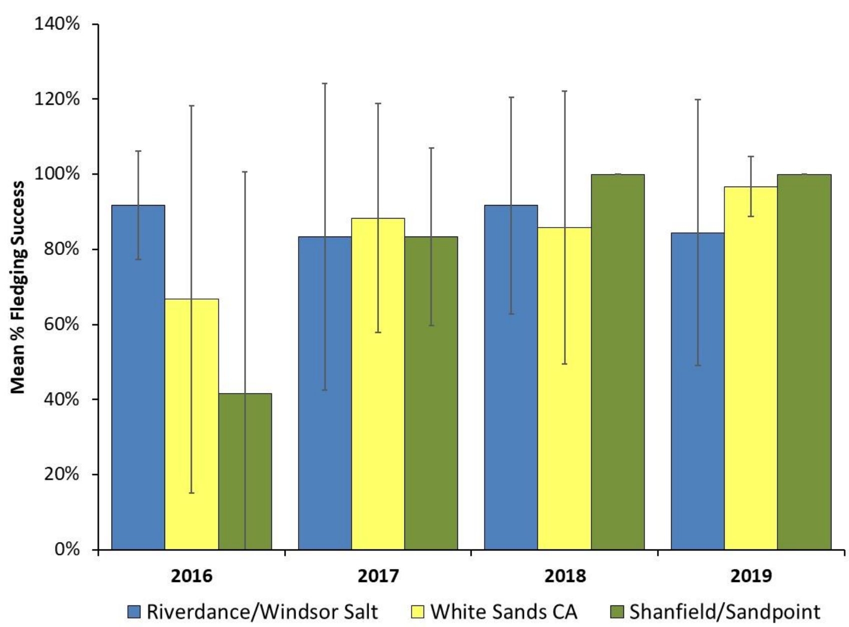
Hatching success of tree swallow eggs at the AOC sites in the four study years ranged, as means, from 80% at Riverdance/Windsor Salt in 2016 to 92% at White Sands CA in 2017 (**Figure 15a**). Following low occupancy of boxes at Riverdance Park, and its relatively close proximity to the Windsor Salt site, the two sites were combined for spatial comparisons. Mean hatching success at Shanfield/Sandpoint, as the reference site, ranged from 92%–100% during these years. No significant differences in hatching success were found among sites in each of the four study years. Clutch size was at least five eggs on average per nest at all sites and study years (data not shown).

Figure 15. Mean hatching success (a) and fledging success (b), as percentages (SD), in tree swallows from three sites in the Detroit River AOC at Riverdance Park/Windsor Salt, White Sands CA, and the upstream reference site at Shanfield/Sandpoint from 2016–2019.

a)



b)



Fledging success at Riverdance/Windsor Salt, White Sands CA and the upstream reference site were, as means, above 83% in all four years of study (**Figure 15b**). Two exceptions were at White Sands CA in 2016 when mean fledging success was relatively lower (67%) following evidence of snake predation of chicks at two nest boxes; nest guards were installed on nest box posts later in the year to remedy this issue. Low fledging success (42%) at Shanfield/Sandpoint in 2016 was associated with competition with nesting house sparrows (*Passer domesticus*). Fledging success was higher in subsequent years at these sites. Overall, there were no significant differences in fledging success among sites in each of the four study years.

Contaminants in Tree Swallow Eggs

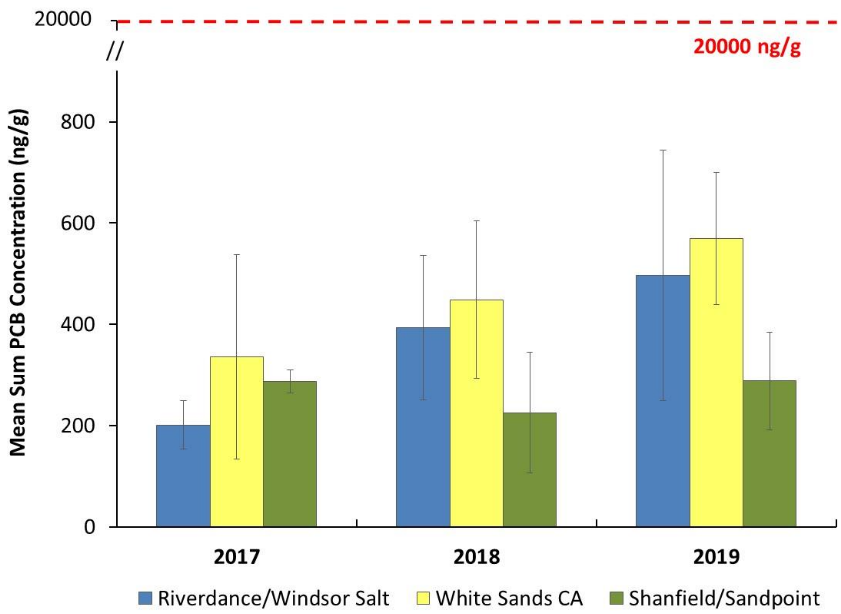
Eggs were collected from study sites from 2017–2019 and analyzed for contaminants. Two eggs per clutch were randomly selected from nests containing at least three fresh eggs and eggs were sampled from five nests per site where possible.

Mean sum PCB concentrations in eggs from all study sites ranged from 200 ng/g at Riverdance/Windsor Salt in 2017 to 570 ng/g at White Sands CA in 2019 (**Figure 16a**). The maximum sum PCB concentration was found in an egg sample from Windsor Salt in 2019 (1140 ng/g). There were no significant differences in sum PCB concentrations in tree swallow eggs among study sites in each of the three study years. This suggests that there was no evidence of increased exposure to sum PCBs in tree swallows at the Riverdance/Windsor Salt and White Sands CA compared to the upstream reference site at Shanfield/Sandpoint. Concentrations of sum PCBs in eggs from all study sites were well below the threshold of 20000 ng/g above which adverse effects on hatching success have been reported in tree swallows (Custer *et al.* 2003).

Mercury concentrations ranged, as means, from 0.034 µg/g in eggs from White Sand CA in 2018 to 0.205 µg/g in eggs from Riverdance/Windsor Salt in 2019 (**Figure 16b**). Significant spatial patterns were found for mercury in eggs among study sites in two of three study years. Mean mercury concentrations were significantly higher in eggs from Riverdance/Windsor Salt compared to White Sands CA while mercury concentrations at the upstream site were statistically similar to these two sites in 2018 and 2019. The maximum mercury concentration was found in an egg sample from Riverdance Park in 2019 (1.35 µg/g) and was an order of magnitude greater than the next highest mercury concentration (0.11 µg/g, egg sample from Windsor Salt). This suggests some variability in exposure to mercury for tree swallows at this site. With the exception of the Riverdance Park sample, mercury concentrations in eggs were well below those associated with adverse reproductive effects in small to medium-sized birds (0.6–2.7 µg/g; Fuchsman *et al.* 2017). There was no evidence of significant effects on hatching success or fledging success in the Riverdance Park clutch from which eggs, with the highest mercury concentration, were collected.

Figure 16. Mean concentrations (SD) of sum PCBs (a) and total mercury (b) in tree swallow eggs from from three sites in the Detroit River AOC at Riverdance/Windsor Salt, White Sands CA, and the upstream reference site at Shanfield/Sandpoint. Threshold concentrations (dotted red lines) indicate concentrations in eggs above which a potential adverse impact on reproduction might be expected. Different uppercase letters indicate significant differences in concentrations between sites within a study year.

a)



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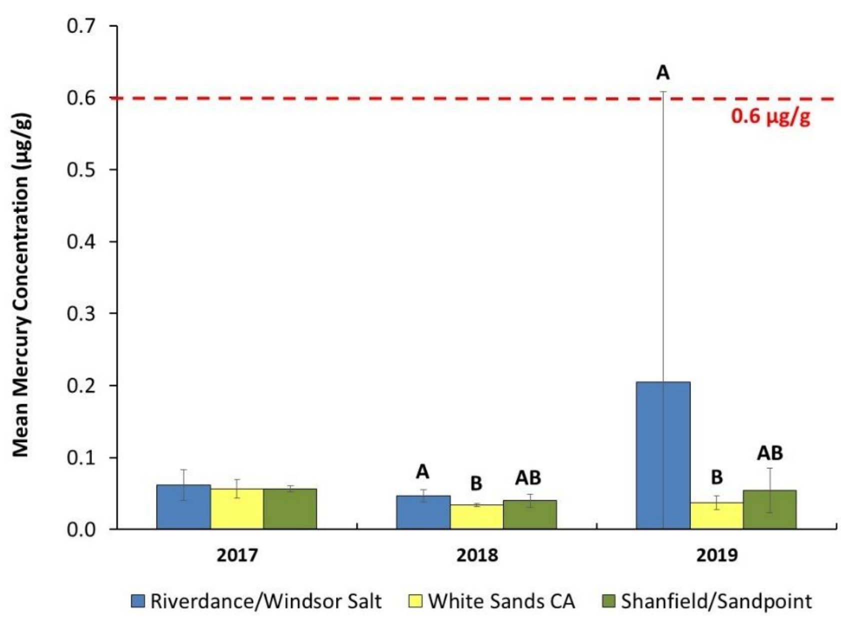

8. Conclusions

Several wildlife studies were conducted between 2008–2019 to determine the status of Beneficial Use Impairment (BUI) #5 - *Bird or Animal Deformities or Reproduction Problems* in the Detroit River AOC (Ontario). The use of several bioindicator wildlife species each with different important attributes (e.g., trophic position, food sources) provides a comprehensive examination of the potential for reproductive problems for the wildlife in the AOC and possible links to exposure of historical legacy contaminants associated with degradation of the aquatic environment in the AOC. The following two delisting criteria were used to assess the status of BUI #5:

Criterion 1: *When incidence rates of bird and animal reproductive problems in sentinel wildlife species do not exceed background levels at suitable reference sites elsewhere in the Great Lakes basin or suitable inland control populations for a minimum of three years; and*

Criterion 2: *When scientifically defensible wildlife bioassays of indicator species confirm that there are no reproductive problems and no significant toxicity from the water column or sediment contaminants or bioaccumulation.*

Reproduction in wildlife was assessed at two important life stages both in the laboratory and in the field. One critical component of successful reproduction is the ability of fertilized eggs to develop and hatch successfully. Artificial incubation of eggs in the laboratory assesses intrinsic factors such as contaminants that may induce early mortality of embryos. In these studies, embryonic viability in cormorants and hatching success of turtle eggs were similar between incubated eggs collected in the AOC and those from corresponding reference sites in all years of study. In frogs, exposure studies were designed to assess the effects of water and sediment quality on the ability of frog eggs to hatch successfully and become free-swimming tadpoles. These studies demonstrated that hatching success of frog eggs was similar between AOC and reference sites following exposure of eggs to water and water and sediment collected from several locations, with varying degrees of contamination, on the River. For birds, in addition to successful hatching of eggs, the ability of nestlings to grow and successfully fledge from the nest is also critical measure of breeding success. Field studies of nesting birds examined hatching success and fledging success (productivity) under real-world conditions in which several stressors may work in concert to adversely impact reproductive success. Hatching success and productivity of tree swallows and black-crowned night-heron nesting at AOC sites were similar to those at upstream reference sites in five of six study years. The single exception was for night-heron at the AOC colony in which reduced reproductive success in 2009 was likely due to extrinsic factors (e.g., predation) and not contaminant-inducted effects in that year. Productivity estimates for herring gulls at the two surrogate AOC colonies downstream in two years were sufficiently high to maintain a stable population. While beyond the boundary of the AOC in western Lake Erie, this area is under the direct influence of historical point sources of contaminants that were discharged in the AOC. Other parameters associated with reproduction measured in night-heron, tree swallows, and herring gulls (e.g., clutch size, clutch volumes) were also similar between study sites.

There is little evidence to suggest that exposure to legacy contaminants originating from localized sources in the AOC were sufficiently high to adversely impact wildlife reproduction in the AOC.

Concentrations of PCBs and mercury in frogs (whole bodies), turtle (eggs) and birds (eggs and nestling liver) in the large majority of samples were below those associated adverse impacts on reproduction. In the few cases, egg samples for tree swallows (2019) and night-heron (2011) exceeded reproduction thresholds for mercury. While it is not known what more subtle effects might be evident at these concentrations, hatching success and fledging success, as the primary measures of assessment here, were not reduced in these years. Despite no evidence of reproductive impairment due to contaminants in this study, it remains unknown what factors contributed to the abandonment of the Canadian side of Detroit River as a breeding site for black-crowned night-heron, herring gulls and ring-billed gulls in the last ten years.

Large declines in PCB and other organochlorine concentrations in herring gull eggs between 1978–2010 suggest that exposure to these compounds has also decreased for birds foraging in the AOC. Concentrations of sum PCBs in cormorant eggs from Shag Island from 2015 –2019 also align well with historical trends reported for gull eggs. These temporal patterns are associated with the restrictions in use and production of these compounds as well as effectiveness of remediation activities in the AOC. At a more local level, concentrations of sum PCBs in turtle eggs also decreased significantly between 2001/02 and 2014/15 at sites downstream on Turkey Creek following remediation at a known site of PCB contamination. These trends provide additional support for the utility of these species as biomonitors of environmental contamination and evidence of improved environmental conditions for wildlife in the AOC.

9. Recommendation for Status Change

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

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Appendices