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

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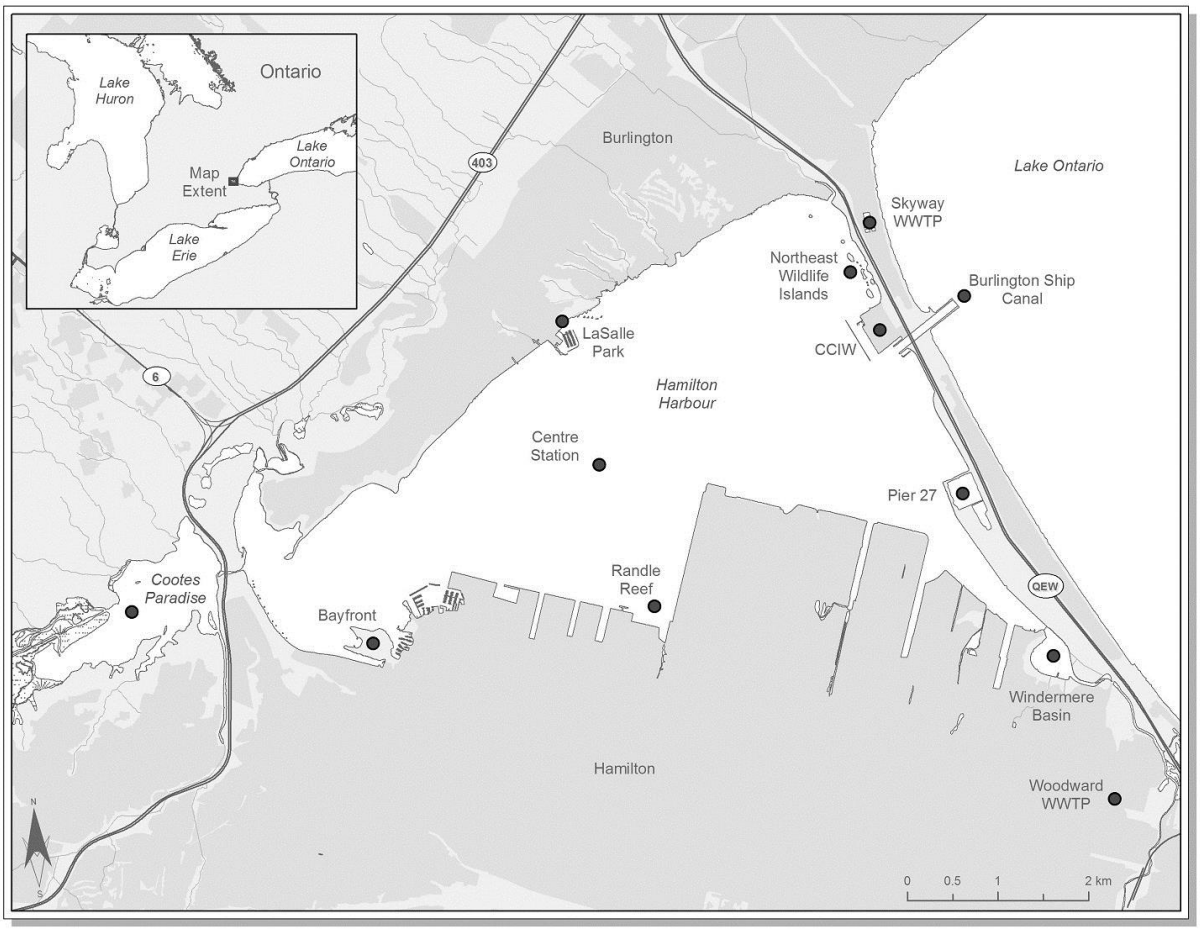
ABSTRACT

Reproduction and development were examined in herring gulls (*Larus argentatus*) breeding within the Hamilton Harbour Area of Concern in 2013, 2015, and 2016. Freshly-laid eggs were collected from one colony within the Area of Concern (AOC) and three reference colonies outside of the AOC, artificially incubated in the laboratory and assessed for embryonic viability, incidence of embryonic deformities, contaminant burdens and other biochemical endpoints (i.e., stable isotopes). Productivity was determined at the colonies when chicks were approximately 21 days old and chicks were examined for morphological deformities as well as other biological endpoints. A second aquatic-feeding colonial waterbird species, the double-crested cormorant (*Phalacrocorax auritus*), nesting at colonies within the AOC, was also selected for assessment of contaminant burdens in 2015 and 2016. Overall, embryonic viability of herring gulls was high (100%) at the AOC colony and herring gull productivity exceeded levels required to maintain a stable population. No embryonic deformities were evident in gull eggs incubated in the laboratory and no morphological deformities were found in 21-day-old herring gull chicks from the AOC colony. Furthermore, a low prevalence of morphological deformities (0.04%) was found in cormorant chicks at two AOC colonies in 2013 and frequencies were not significantly different from the Lake Erie reference colony. Significantly higher concentrations of two persistent organochlorines, sum PCBs and *p,p'*-DDE, were found in gull embryos from the AOC colony compared to one reference colony in the two study years, a pattern likely due to several factors specific to the Harbour. Mercury concentrations in gull embryos were similar or lower than concentrations at reference colonies. Concentrations of organochlorines and mercury were largely similar between herring gulls (embryos) and cormorants (eggs), two colonial waterbird species with a close connection to the aquatic environment. Large declines in concentrations of sum PCBs and other organochlorines, mercury, and 2,3,7,8-TCDD in herring gull eggs since the 1980s indicate that exposure to these compounds has decreased in herring gulls foraging in the AOC. Large decreases in organochlorine burdens were also found in double-crested cormorant eggs between 1989 and 2016. Two biochemical endpoints relating to growth and development were not adversely impacted in juvenile chicks from the AOC colony and immune function was not significantly different between juvenile chicks from the AOC colony and the Lake Ontario reference colony. In summary, concentrations of PCBs and other organochlorine compounds, PBDEs, dioxins and furans, and mercury were not sufficiently elevated to adversely impact the reproductive success and development of herring gulls and cormorants nesting in the Hamilton Harbour AOC.

INTRODUCTION

The Hamilton Harbour Area of Concern (AOC) is one of 43 Great Lakes AOCs that were initially identified by Canada, the United States and the International Joint Commission (IJC) as specific locations where local environmental degradation had severely impacted the area's ability to support aquatic life. Located at the western tip of Lake Ontario, Hamilton Harbour is a 2150 hectare bay that is connected to Lake Ontario by a narrow shipping channel. The AOC is comprised of Hamilton Harbour and Cootes Paradise covering an area of approximately 24 km^2 (Figure 1) which drains a large and extensive watershed approximately 500 km^2 in size. Historical discharges of pollutants from local industries and wastewater treatment plants, combined sewer overflows, urban and rural runoff, atmospheric and agricultural

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



loadings, contaminant spills and leachate from landfills impaired water quality and contaminated sediment in the Harbour (Hamilton Harbour Remedial Action Plan 1992). Several contaminants of concern were identified in the AOC including polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), mercury and other metals, which contributed to exceedances of water quality objectives, sediment quality guidelines and/or fish consumption guidelines where these were available (Hamilton Harbour Remedial Action Plan 1992).

Fourteen beneficial use impairments (BUIs) were used by the Canadian and U.S. federal governments to identify and assess the extent of environmental degradation at Great Lakes AOCs and thereby direct restoration and remediation activities. One of these BUIs, "bird or animal deformities or reproduction problems", addresses contaminant exposure or other anthropogenic environmental stressors on reproductive success or deformity rates in wildlife. This was an issue in Hamilton Harbour in 1970 when low hatchability was reported in common terns (*Sterna hirundo*) nesting on two islands, formerly known as the Hydro Islands (Connors *et al.* 1975). Subsequent collections of unhatched eggs at the end of the breeding season indicated that these eggs were contaminated with several organochlorines including PCBs (Gilbertson and Reynolds 1972). Collections of tern eggs over the breeding season in 1971 showed that concentrations of these compounds increased as laying date after tern arrival increased, suggesting

that terns were accumulating contaminants acquired in their local foraging area, including Hamilton Harbour, over the nesting season (Gilbertson 1974a). Deformities were also found in three tern chicks of 240 chicks examined in the Harbour in 1971 and 1972 as part of colonial waterbird surveys conducted on the lower Great Lakes and at a time when contaminants were suspected to be the cause of these abnormalities (Gilbertson *et al.* 1976). In the late 1980s, elevated frequencies of unhatched eggs and hatchling deformities were found in snapping turtles (*Chelydra serpentina*) from Cootes Paradise where organochlorine burdens in eggs were also high (Bishop *et al.* 1991). There was also evidence of alterations in a sexually dimorphic trait in male and female adult turtles from Cootes Paradise in the mid-1990s (de Solla *et al.* 1998). The official status of the BUI was changed from "impaired" in 2002 to "requires further assessment" in 2012 following refinements to the delisting objective (Hamilton Harbour Remedial Action Plan Stakeholder Forum 2012). In 2013, studies of the potential effects of contaminants on reproduction and development in colonial waterbirds were initiated by Environment and Climate Change Canada (ECCC) to more fully evaluate and assess the current status of this BUI in the Hamilton Harbour AOC. The approach used here has been implemented in the St. Marys River (Ontario) and Thunder Bay AOCs for similar assessments of this BUI.

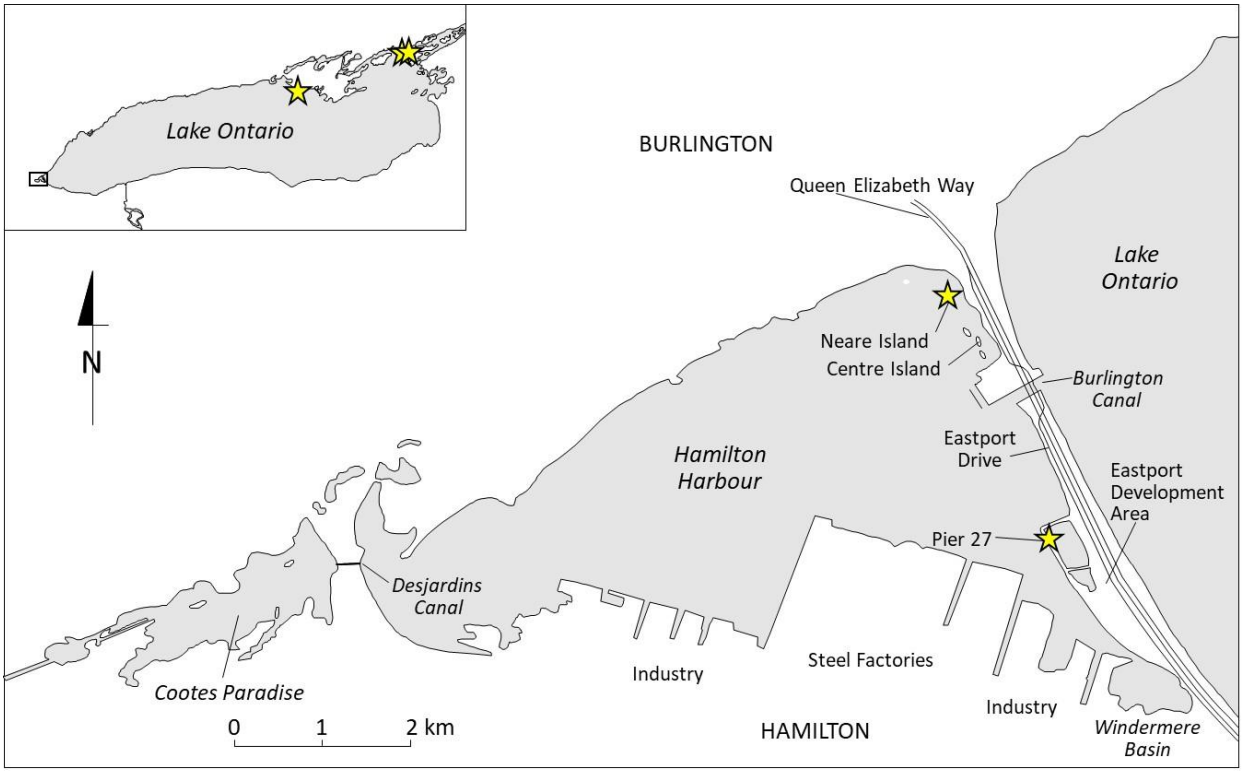
Fish-eating wildlife, such as colonial waterbirds, are important indicators of exposure to persistent contaminants in the aquatic environment (Fox and Weseloh 1987). As top predators, they occupy a high trophic level in the aquatic food web and therefore can accumulate high levels of contaminants, which may in turn adversely affect their reproductive health and development. Two colonial waterbird species that breed and forage within the Hamilton Harbour AOC were selected for assessment purposes. The herring gull (*Larus argentatus*) is a long-lived, primarily fish-eating colonial waterbird that, from the time it reaches breeding age, is a year-round resident in the Great Lakes basin. The herring gull has been used as an avian sentinel species in the Great Lakes for decades. A second aquatic-feeding colonial waterbird species, the double-crested cormorant (*Phalacrocorax auritus*), was selected for assessment of contaminant burdens. This species feeds almost exclusively on fish compared to herring gulls that are opportunistic feeders and will consume terrestrial prey if fish are not readily available. This close connection to the aquatic environment is vital for assessment of local conditions in the AOC.

In 2013 and 2015, breeding colonies of herring gulls were studied by ECCC in the Hamilton Harbour AOC. Freshly-laid herring gull eggs were collected for artificial incubation in the laboratory to assess embryonic viability, incidence of embryonic deformities, contaminant burdens and biochemical endpoints (i.e., stable isotopes). Under controlled laboratory conditions, this method assesses the effects of embryonic exposure to potentially high levels of contaminants during critical periods of development. Reproduction and development were also assessed in wild populations in 2013, 2015 and 2016 with visits to colonies to monitor productivity and examine gull chicks for morphological deformities and to measure additional biochemical endpoints (i.e., stress hormone, thyroxine levels) relating to growth and development as well as immune function that could be affected by increased contaminant exposure. Surveys of morphological deformities in cormorant chicks were also conducted at AOC colonies in 2013. The results of this study will be used to assess the status of the wildlife reproduction and deformities BUI in this AOC.

METHODS

One herring gull colony at Neare Island (43°18’30”N, 79°48’20”W) in the Hamilton Harbour AOC was selected for study purposes in 2013, 2015 and 2016 (Figure 2). This island is approximately 0.13 hectares in size and is situated in the northeastern corner of Hamilton Harbour. Scotch Bonnet Island (43°53’59”N, 77°32’30”W), situated in the eastern basin of Lake Ontario and approximately 0.92 hectares in size, was selected as the herring gull reference colony (Figure 2). Due to the displacement of nesting gulls by double-crested cormorants that had been disturbed following cull operations at nearby Presqu’ile Provincial Park, it was not possible to conduct the field component of this study at Scotch Bonnet Island in 2013. Consequently, an alternate reference colony at Double Island (46°10’24”N, 82°51’50”W) in the North Channel of Lake Huron was used for productivity estimates and field assessments of chicks in this year. Double Island has served as the non-AOC reference site for similar assessments of other Great Lakes AOCs and this coincided with work that was already underway there. In 2015, Salmon Island (44°11’55”N, 76°35’32”W) was selected as the alternate eastern Lake Ontario reference colony for the artificial incubation study and field studies (Figure 2). Field data collected at the Double Island colony in 2015 are provided where available.

Figure 2. Colony locations for herring gulls and double-crested cormorants in the Hamilton Harbour AOC in 2013, 2015 and 2016. Yellow stars in the inset represent Lake Ontario reference colonies, from west to east, Scotch Bonnet Island, Salmon Island, and Snake Island. Map has been modified from Pekarik *et al.* (1997).



Visits to each gull colony were made at two times during the breeding season: 1) egg laying (late April) and 2) when chicks were 21 days old (mid-May) in 2013, 2015 and 2016 to assess reproduction and various parameters of health. During the first visit in 2013 and 2015, 15 freshly-laid eggs (i.e., not incubated) were collected from one-egg nests at each colony for artificial incubation in the laboratory at the National Wildlife Research Centre (NWRC) in Ottawa. Embryonic viability, incidence of embryonic deformities, contaminant burdens, and stable isotope signatures were determined. In addition, a thorough nest count of the entire colony was conducted and contents of each nest were recorded. Individual nest enclosures (~1m in diameter and 40 cm high) were constructed around ten or twelve 3-egg nests at each colony. As a measure of colony health, egg measurements for up to forty-two 3-egg clutches were recorded (in millimetres) and egg volume calculated as:

$$\text{Egg volume } (cm^3) = 0.489 \times (\text{length} \times \text{breadth}^2)/1000$$

Total clutch volume was determined as the sum volume of the three eggs in the clutch. Intraclutch variation in egg size was calculated as the difference in volume between the largest and smallest egg in the clutch divided by the largest egg size (i.e., volume) and multiplied by 100.

During the second visit, when chicks from enclosed nests were approximately 21 days old, productivity was calculated as:

$$\text{Productivity} = \text{no. of 21-day-old chicks/no. of enclosed nests}$$

Enclosed nests that had been abandoned, damaged, or where there was evidence that chicks had escaped were not included in estimates of productivity. Body measurements of chicks, including mass, tarsus, wing cord, and culmen length were recorded and chicks were banded with a stainless steel USFW band. Chicks were assessed for morphological deformities and overall health. A blood sample was collected from the brachial vein of chicks using a 25 gauge x 5/8" needle and heparinized syringe to examine thyroxine concentrations in plasma (see below for further details). In addition, two secondary covert feathers were collected to quantify corticosterone concentrations as a measure of stress over time in herring gull chicks. Immune status of chicks was evaluated using a phytohemmagglutinin (PHA) skin test.

In 2016, a third year of field study was initiated that included an additional AOC herring gull colony at Pier 27 (43°17'1"N, 79°47'37"W), one of three piers that comprise the Eastport Development Area, as well as at Neare Island (Figure 2). Egg measurements were recorded at both nesting colonies at Neare Island and Pier 27. However, it was not possible to estimate productivity at these two colonies since erected enclosures were either knocked down by nesting cormorants or abandoned. For the few enclosures that were not compromised at Neare Island, health parameters were examined in juveniles as well as at reference colonies (Salmon Island and Double Island) to supplement the two earlier years of data collected for corticosterone and thyroxine and one year of data collected for the immune status test in 2015.

Double-crested cormorant eggs were also collected for assessments of contaminant burdens in 2015 and 2016. Cormorant colonies studied in the Hamilton Harbour AOC were at Centre Island (43°18'18"N, 79°48'11"W) in both study years and Pier 27 which was added as a second AOC colony in 2016 (Figure 2). Snake Island (44°11'27"N, 76°32'35"W) in eastern Lake Ontario was the reference colony in both

study years (Figure 2). Colony visits were conducted in May when 12 or 13 eggs were collected from nests containing $\geq$ 3 eggs. After collection, the eggs were sent to NWRC where contents were placed in chemically-cleaned glass jars, homogenized, and frozen until chemical analysis. Eggs from each colony were pooled together as a single sample for chemical analysis.

Artificial Incubation of Herring Gull Eggs:

In 2013 and 2015, unincubated herring gull eggs were collected in the field from nests containing a single egg, transported to NWRC in insulated coolers with foam inserts and set in a Petersime incubator (model# MX-1) at 37^{o}C, 58% humidity 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. Each embryo was examined for physical deformities. 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. Eggs that were nonviable were staged if possible (e.g., infertile; early, mid or late embryo death). Egg contents, including yolk sac, whole carcass, and shell membranes, were collected in chemically-cleaned glass jars, homogenized, and frozen until chemical analysis for contaminants. Ten or fifteen embryos were randomly selected from each colony for chemical analysis in the two study years.

Contaminant Analyses:

Chemical analyses of herring gull embryos and cormorant eggs for organochlorine compounds and polybrominated diphenyl ethers (PBDEs) were conducted at the Great Lakes Institute for Environmental Research at the University of Windsor (2013 and 2015-AOC and Lake Ontario reference gull colonies) and at NWRC (2013-Lake Huron reference gull colony, 2015 and 2016-AOC and reference cormorant colonies). Organochlorine compounds measured included *p,p'*-DDE (dichlorodiphenyldichloroethylene), oxychlordane, *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, hexachlorobenzene (HCB), dieldrin, heptachlor epoxide (HE), mirex, octachlorostyrene (OCS), and polychlorinated biphenyls (PCBs). Sum chlordane is based on the sum concentrations of oxychlordane, *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, and *trans*-nonachlor. Prior to chemical analysis, thawed embryos and eggs were homogenized and then underwent neutral extraction and removal of lipids and biogenic compounds by gel permeation chromatography and further clean up by Florisil column chromatography. Quantitative analysis of organochlorine compounds was performed using capillary gas chromatography coupled with a mass selective detector (GC-MSD) operated in selected ion monitoring mode. PBDEs were quantified by gas chromatography high resolution mass spectrometry methods using a time-of-flight mass spectrometer (GC-MS-TOF) at GLIER and by GC-MSD operated in the NICI mode at NWRC. Sum PCBs were based on the sum concentrations of 32–62 individual or co-eluting PCB congeners found above the limit of detection depending on the year and laboratory performing the analysis. Similarly, sum PBDEs were based on the sum concentrations of 13–15 individual or co-eluting PBDE congeners found above the limit of detection. Certified internal standards were used for quantification and certified reference materials, blanks and duplicate samples were analyzed for quality assurance purposes. Concentrations of organochlorines and PBDEs are reported in µg/g on a wet weight basis. Detection limits for organochlorine compounds and individual PBDE congeners ranged from 0.00001–0.005 µg/g.

Herring gull embryos were analyzed in 2015 for non-*ortho s*ubstituted PCBs, polychlorinated dibenzo-*p*-dioxins, including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and polychlorinated dibenzofurans using gas chromatography high resolution mass spectrometry (GC/HRMS) at RPC Laboratory in Fredericton, New Brunswick. Methods were based on US EPA Method 1613B and 8290A for dioxins and furans and on US EPA Method 1668C for non-*ortho* PCBs. Reference materials, blanks, and duplicates were analyzed for quality assurance purposes. Samples were analyzed as a single pool consisting of 15 gull embryos at each colony.

Total mercury was quantified at NWRC on a dry weight basis using an Advanced Mercury Analyzer (AMA-254) as described in CWS Method No. MET-CHEM-AA-03J and MET-CHEM-THg-01A in 2013 and 2015, respectively. Certified reference materials and duplicate samples were also analyzed to ensure correct calibration, accuracy, and reproducibility of test methods. Mercury concentrations in embryos and eggs are reported in µg/g on a wet weight basis using percent moisture content.

Stable Isotopes:

Stable isotope analyses of samples were conducted at the University of Ottawa's G.G. Hatch Stable Isotope Laboratory in Ottawa, Ontario. Following lipid-extraction, samples were weighed into tin capsules and loaded into an elemental analyser. The sample was flash combusted at ~1800^{o}C (Dumas combustion) and the resultant gas products were carried by helium through columns of oxidizing/reducing chemicals optimised for CO_2 and N_2. The gases were separated by a purge and trap adsorption column and then sent to the Delta Advantage isotope ratio mass spectrometer coupled with Conflo III. Samples were normalized to internal standards and calibrated to international standards. Stable isotope ratios are expressed in δ notation as the deviation from standards in parts per thousand (‰) according to the following relationship:

$$\delta X = (R_{sample}\text{-}R_{standard})/R_{standard} \times 1000$$

where X is ^{15}N or ^{13}C and R is the corresponding ratio $^{15}N/^{14}N$ or $^{13}C/^{12}C$. In this study, $\delta^{15}N$ signatures were compared to infer relative (and not absolute) trophic position at colonies.

Feather Corticosterone:

Feathers from chicks were prepared by first removing the calamus, i.e., the proximal end of the quill to where the feathers start (~ 5 to 10 mm, depending on length of feather). The remaining portion was then minced with scissors until homogenous and 1.5 ml methanol was added. The sample was vortexed, sonicated for 30 minutes at room temperature, and incubated overnight at 50^{o}C while shaking. After centrifuging at 13,000 rpm for 20 minutes, the supernatant was transferred to a fresh tube and the original feather sample was re-extracted with 1 ml methanol. This re-extraction was again vortexed for 5 minutes and the supernatant was removed and added to the original fraction. The entire methanol fraction was evaporated to dryness overnight in a fume hood and then the sample was reconstituted with 200 µl steroid diluent.

Corticosterone concentrations in the extracts of the feather sample were determined using a corticosterone EIA kit (Assay Designs – corticosterone enzyme immunoassay kit; product no. 900-097; 96 well kit). This is a competitive immunoassay for the quantitative determination of corticosterone in

biological fluids and it uses a polyclonal antibody to corticosterone to bind in a competitive manner. In the presence of the corticosterone antibody, corticosterone in the sample competes with a known amount of corticosterone that has an alkaline phosphatase molecule covalently attached to it. The excess reagents are washed away and the substrate is added. Following a short incubation time, the enzyme reaction is stopped and the samples are read on a SpectraMax 190 UV-VIS microplate reader (Molecular Devices, Sunnyvale, California, USA) at 405 nm. The intensity of colour produced in the reaction is inversely proportional to the concentration of corticosterone in the sample. The measured optical density was used to calculate the concentration of corticosterone. The average net optical density (OD) bound for each standard and sample is calculated by subtracting the average NSB (non-specific binding) OD from the average OD bound:

Average Net OD = Average Bound OD - Average NSB OD

Then the binding of each pair of standard wells as a percentage of the maximum binding wells (Bo) was calculated using the following formula:

Percent Bound = Net OD x 100 Net Bo OD

The plot of percent bound versus concentration of corticosterone for the standards was graphed using Prism software (GraphPad, La Jolla, California, USA) and a line fitted through the points. The concentration of corticosterone in the sample was then determined. An in-house quality control sample was used in each plate. The sensitivity of the assay is 27 pg/ml.

Thyroid Status:

Blood samples were centrifuged for 5 minutes at 14,000 rpm to separate plasma from red blood cells. Plasma was stored at -80°C and red blood cells were stored at 4°C. Concentrations of free thyroxine in the plasma of chicks were determined using a commercially available kit (AccuBind Elisa microwells product no. 1225-300 - Monobind Inc., Lake Forest, CA, 92630, USA). The method is based on a competitive enzyme immunoassay format in which a competition is set up between an immobilized antibody, the enzyme-antigen conjugate and the free thyroxine in the sample. When equilibration is reached, the unbound antigen fraction is removed and the enzyme activity in the bound fraction is measured which is inversely proportional to the concentration of free thyroxine in the sample. As per the method, standards, controls and sample plasma, as well as the enzyme reagent, were added to the microplate wells, which contained the immobilized antibody. After an incubation period, the unbound fraction was washed from the wells and the substrate added. The reaction was stopped and the plate was read at 450 nm on a SpectraMax 190 UV-VIS microplate reader (Molecular Devices, Sunnyvale, California, USA). Concentrations were determined from the standard curve, which was fitted using a variable slope (four parameter) method using Prism software (GraphPad, La Jolla, California, USA).

Controls used were Randox product no. HS2611 assayed human sera levels 2 & 3 (Randox Laboratories Ltd., Antrim, UK). The method gave the intra-assay precision as 10.98%, 4.26%, and 3.25 % coefficient of variation for low, medium and high controls, respectively. The inter-assay precision was 10.81%, 6.01%, and 7.90 % coefficient of variation for low, medium, and high controls, respectively. The detection limit of this assay was 0.32 ng/dl.

Immune Response - Phytohemagglutinin (PHA) Skin Test:

Feathers were plucked from the patagium ("wing web") of each wing in order to measure the thickness to the nearest 0.05 mm using pressure-sensitive calipers (Dyer 304-196). Two measurements were taken of each wing web and the mean of the two measurements was determined for each wing. To assist in measuring the same location on day 2, a small dot was made with a permanent marker at the site of measurement. Following the thickness determination, 0.1 ml of a 1 mg/ml solution of PHA (Sigma) dissolved in sterile phosphate buffered saline (PBS; 0.01M phosphate buffer, 0.137M NaCl, pH 7.4) was injected intra- or sub-dermally into one wing web and 0.1 ml of PBS solution was injected into the other wing web as a control using a 1 ml syringe fitted with a 26 gauge intradermal needle. Injections of PHA and PBS were randomly alternated between right and left wings of birds tested. Twenty-four hours ($\pm$6 hours) after injection, the thickness of each wing web was re-measured in duplicate at the site of injection (indicated by marker dot) and the mean of the two measurements again determined. For each individual chick, the PHA stimulation index was calculated as the increase in wing web thickness caused by PHA in one wing web minus the increase caused by PBS in the other wing web. This test was conducted using chicks at study sites in 2015 and 2016 only.

Field Survey of Deformities in Cormorants:

Field surveys of morphological deformities in cormorant chicks were conducted in 2013 at AOC colonies at Centre Island and Pier 27 and a reference colony at Mohawk Island National Wildlife Area (42°50'5"N, 79°31'23"W) located in eastern Lake Erie. Mohawk Island was surveyed on June 12th by boat, and Centre Island and Pier 27 on June 20th, by boat and by land, respectively. Since adult cormorants flush at the approach to the nests, only chicks or eggs remained on the colony during sampling. Starting from one end of the colony, nests with chicks were visited, and chicks were assessed for deformities, and placed back into their nests immediately after evaluation (usually within a minute or less per chick). Chicks were evaluated for gross morphological deformities to their bills, head, wings, and feet, and pictures were taken of any abnormal development. Nests were marked to prevent multiple sampling. During the colony survey, a long range water pistol (e.g. Super Soaker™) was used to scare off herring gulls from depredating chicks or eggs when the adult cormorants were scared off their nests. After the sampling was complete, the surveyors left the area to allow the adult cormorants to return to their nests.

Statistical Analysis:

Contaminants and other biological endpoints were statistically analyzed using either the Student's *t*-test for between colony comparisons or a one-way ANOVA for among-colony comparisons, which when significant, was followed by Tukey's HSD test. Data were log-transformed (log_{10}) to meet conditions of equal variance and normality for parametric analysis. If data failed these assumptions, comparisons were made using either a Mann-Whitney U non-parametric test or Kruskal-Wallis one-way analysis of variance by ranks; post-hoc tests were conducted using non-parametric multiple comparison tests for unequal sample sizes. Concentrations of chemical and biochemical endpoints that were below the limit of detection were given a value of one-half of the detection limit. Since numbers of PCB and PBDE congeners differed among gull colonies in 2013 (due to different laboratories performing chemical analyses), statistical analysis of contaminant burdens among colonies were conducted using congeners

that were common to all chemical analyses. For sum PCBs and sum PBDEs, this represented 32 and 13 common congeners, respectively. Mercury concentrations in samples were statistically analyzed on a dry weight basis; however, concentrations are reported on a wet weight basis for comparisons to published values and thresholds. A Spearman rank correlation analysis was performed to examine the relationship between the two stable isotopes in gull embryos. All results were considered significant at $p<0.05$.

Concentrations of 2,3,7,8-TCDD toxic equivalents (TEQs) were calculated for dioxin-like PCBs, furans, and dioxins and are based upon toxic equivalency factors developed by van den Berg *et al.* (1998) for birds. Dioxin-like PCBs include four non-*ortho* PCB congeners (77, 81, 126, and 169) and eight mono-*ortho* PCB congeners (105, 114, 118, 123, 156, 157, 167, and 189). Three of the eight mono-*ortho* PCB congeners, i.e., 105, 118, and 156, were quantified in chemical analyses for organochlorines for which embryos were analyzed as individuals. Mean concentrations for these three PCB congeners were calculated for reporting of TEQs associated with these dioxin-like PCBs in the pooled samples. Total TEQ concentration is based on the sum concentration of TEQs calculated for the 4 non-*ortho* PCBs, 3 mono-*ortho* PCBs, and 17 dioxin and furan congeners.

RESULTS

A) Artificial Egg Incubation Study

Embryonic Viability and Deformities:

Embryonic viability was consistently high at 100% in herring gulls from the Hamilton Harbour AOC colony at Neare Island in 2013 and 2015 (Table 1). Embryonic viability was also high overall at 96% in gulls from the two Lake Ontario reference colonies, Scotch Bonnet Island and Salmon Island, and the Lake Huron reference colony at Double Island in 2013 and 2015. Of 42 fertile eggs examined in 2013, one embryo from Double Island died (stage 36/37). Of 30 fertile eggs examined in 2015, one embryo from Salmon Island died (stages 37/38). This embryo was also deformed and had an edema around the neck. Embryonic deformities were not observed in incubated eggs from the AOC colony in either study year. Overall, artificially incubated herring gull eggs collected from the AOC colony had high embryonic viability and no embryonic deformities, similar to the results observed at the reference colonies in the two study years.

Contaminants:

Of all organochlorine compounds, sum PCBs were found at the highest concentrations in embryos with mean concentrations ranging from 1.28 µg/g in embryos from Double Island in 2013 to 4.03 µg/g in embryos from Neare Island in 2013 (Table 2a). Mean concentrations of the remaining organochlorines in embryos were generally below 1.0 µg/g at study colonies. Concentrations of *p,p'*-DDE in embryos were, based on comparisons of means, at least five times higher than concentrations of the remaining organochlorines. Maximum concentrations of sum PCBs (6.28 µg/g) and *p,p'*-DDE (1.63 µg/g) in this study were found in embryos from the AOC colony and Scotch Bonnet reference colony, respectively, in 2013. The maximum sum PBDE concentration (1.14 µg/g) was found in an embryo from the AOC colony in 2015. Significant differences in concentrations of sum PCBs and *p,p'*-DDE were evident in herring gull

Table 1. Embryonic viability and incidence of embryonic deformities in artificially incubated herring gull eggs collected from the Hamilton Harbour AOC colony (Neare Island) and corresponding reference colonies (Scotch Bonnet Island, Double Island, and Salmon Island) in 2013 and 2015.

Colony	AOC/ Ref	Year	Total No. Eggs	No. Infertile Eggs	No. Fertile Eggs	No. Viable Eggs	No. Dead Eggs	Embryonic Viability (%)	No. Deformities	Deformities (%)
Neare I.	AOC	2013	15	3	12	12	0	100%	0	0%
	AOC	2015	15	0	15	15	0	100%	0	0%
Overall	**AOC**		**30**	**3**	**27**	**27**	**0**	**100%**	**0**	**0%**
Scotch Bonnet I.	Ref	2013	15	0	15	15	0	100%	0	0%
Double I.	Ref	2013	15	0	15	14	1	93%	0	0%
Salmon I.	Ref	2015	15	0	15	14	1	93%	1	7%
Overall	**Ref**		**45**	**0**	**45**	**43**	**2**	**96%**	**1**	**2%**

Table 2. Concentrations of organochlorines and sum PBDEs (µg/g, wet weight) in embryos of herring gulls (a) and eggs of double-crested cormorants (b) from Hamilton Harbour AOC colonies (Neare Island, Centre Island, and Pier 27) and corresponding reference colonies (Scotch Bonnet Island, Double Island, Salmon Island, and Snake Island) in 2013, 2015, and 2016. Mean concentrations (SD) in herring gulls are based on analysis of 10 individual embryos per colony following incubation in the lab. Concentrations in double-crested cormorant eggs are based on analysis of a single pool consisting of 13 eggs at Centre Island in 2015 and at Snake Island in 2015 and 2016 and a mean concentration (SD) at Hamilton Harbour colonies (two pools of eggs from Pier 27 and Centre Island consisting of 12 and 13 eggs, respectively) in 2016. Different uppercase letters indicate significant differences among gull colonies within a study year. Statistical comparisons of sum PCBs and sum PBDEs are based on congeners that were common among colonies.

a) Herring gull embryos:

Colony	AOC/ Ref	Year	*p,p'*-DDE	Sum Chlordane	Dieldrin	OCS	HCB	HE	Mirex	Sum PCBs[a]	Sum PBDEs[b]
Neare I.	AOC	2013	0.973 (0.328) **A**	0.038 (0.012)	0.026 (0.022) **A**	0.002 (0.001) **A**	0.010 (0.002)	0.007 (0.002)	0.084 (0.039) **A**	4.033 (1.307) **A**	0.329 (0.182) **A**
Scotch Bonnet I.	Ref	2013	1.128 (0.397) **A**	0.047 (0.024)	0.013 (0.007) **AB**	0.003 (0.001) **A**	0.013 (0.004)	0.008 (0.003)	0.205 (0.096) **A**	3.275 (1.412) **A**	0.412 (0.204) **A**
Double I.	Ref	2013	0.269 (0.154) **B**	0.039 (0.013)	0.009 (0.006) **B**	0.001 (0.001) **B**	0.013 (0.005)	0.009 (0.003)	0.013 (0.019) **B**	1.280 (1.070) **B**	0.198 (0.111) **B**
Neare I.	AOC	2015	0.671 (0.333) **A**	0.026 (0.012)	0.008 (0.004)	0.003 (0.002)	0.008 (0.005)	0.004 (0.002)	0.095 (0.061)	3.351 (1.398) **A**	0.405 (0.319)
Salmon I.	Ref	2015	0.420 (0.183) **B**	0.021 (0.013)	0.007 (0.005)	0.002 (0.002)	0.011 (0.017)	0.005 (0.003)	0.090 (0.046)	1.473 (0.534) **B**	0.477 (0.366)

b) Double-crested cormorant eggs:

Colony	AOC/ Ref	Year	*p,p'*-DDE	Sum Chlordane	Dieldrin	OCS	HCB	HE	Mirex	Sum PCBs[a]	Sum PBDEs[b]
Centre I.	AOC	2015	1.930	0.021	0.028	0.004	0.012	0.010	0.055	4.083	0.137
Snake I.	Ref	2015	0.440	0.006	0.012	0.002	0.010	0.005	0.022	0.908	0.033
Centre I. & Pier 27	AOC	2016	0.593 (0.013)	0.007 (0.001)	0.019 (0.005)	0.007 (0.007)	0.006 (0.002)	0.004 (0.001)	0.015 (0.015)	1.600 (0.247)	0.050 (0.002)
Snake I.	Ref	2016	0.687	0.015	0.021	0.001	0.007	0.010	0.022	1.982	0.047

[a] Based on sum concentrations of 32–62 PCB congeners

[b] Based on sum concentrations of 13–15 PBDE congeners

embryos among study colonies in the two study years. In 2013, concentrations of these two compounds, as well as dieldrin, OCS, mirex and sum PBDEs, were significantly higher in embryos from the AOC colony compared to the Double Island reference colony but were not significantly different from the Lake Ontario reference colony at Scotch Bonnet Island. In 2015, concentrations of these two compounds were significantly higher at the AOC colony compared to the Lake Ontario reference colony at Salmon Island while concentrations of the other organochlorines and sum PBDEs were similar between the two colonies. Percent lipid content in herring gull embryos in 2013 was significantly higher in embryos from Double Island (mean=7.7%) compared to Neare Island (mean=6.1%; $F_{2,27}$=5.35, p=0.01). As such, the results reported in Table 2 in 2013 are based on data which were first lipid-normalized prior to statistical analysis, but are reported on a wet weight basis. Percent lipid content was not significantly different between colonies in 2015 with means in herring gull embryos of 7.1% and 7.3% at Neare Island and Salmon Island, respectively.

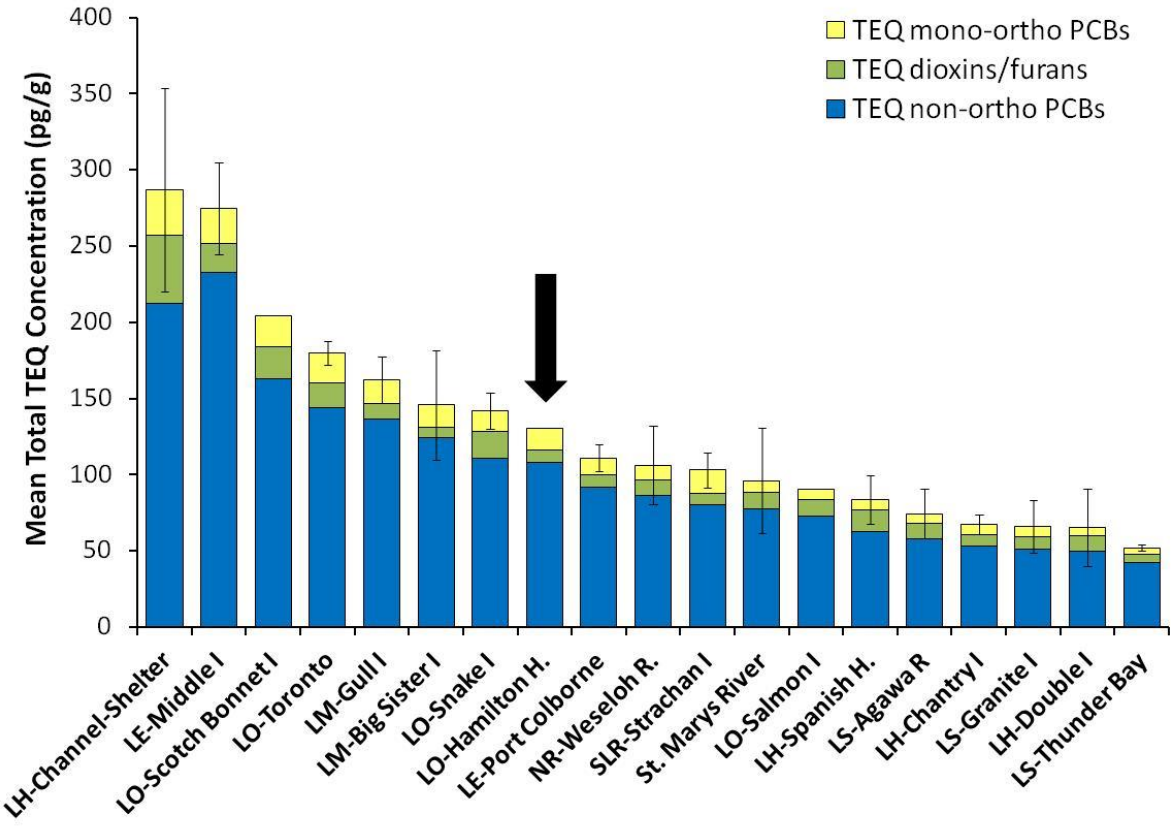
In 2015, concentrations of all organochlorines (including PCBs) and sum PBDEs were consistently higher in double-crested cormorant eggs (as pooled samples) from the AOC colony at Centre Island compared to the Lake Ontario reference colony at Snake Island (Table 2b). This pattern was not evident in 2016 when mean concentrations at the two Hamilton Harbour colonies were more similar to the Snake Island reference colony. Sum PCBs in cormorant eggs were found at the highest concentrations (4.08 µg/g) followed by *p,p'*-DDE (1.93 µg/g), sum PBDEs (0.137 µg/g), and then the remaining organochlorines. Comparing burdens between the two species at AOC colonies, concentrations of all organochlorines and sum PBDEs in cormorant eggs in 2015 and 2016 were within the ranges of concentrations in gull embryos in 2013 and 2015. One exception was *p,p'*-DDE in 2015 when the concentration in the pooled sample of cormorant eggs was 22% higher than the maximum concentration in gull embryos.

Concentrations of four non-*ortho* PCBs were higher in embryos from the Hamilton Harbour AOC colony compared to the Salmon Island reference colony in 2015 (Table 3). Of the four non-*ortho* PCBs measured in herring gull embryos from the AOC colony, concentrations of PCB-126 > 77 > 169 > 81 while rankings of congeners PCB-77 and PCB-169 were reversed in embryos from the reference colony. The concentration of the dioxin congener 2,3,7,8-TCDD was relatively lower in embryos from the AOC colony (5.77 pg/g) compared to those from the Salmon Island reference colony (7.13 pg/g). Higher concentrations of non-*ortho* PCBs and mono-*ortho* PCBs overall in embryos from the AOC colony contributed to the higher total TEQ concentration in AOC embryos (130.60 pg TEQ/g) compared to reference colony embryos (90.44 pg TEQ/g). Toxicity associated with non-*ortho* PCBs, dioxins and furans, and mono-*ortho* PCBs contributed 83%, 6%, and 11%, respectively, to the mean total TEQ concentration in embryos from the AOC colony. Toxicity associated with non-*ortho* PCBs, dioxins and furans, and mono-*ortho* PCBs at the reference colony was slightly different with contributions of 80%, 12%, and 8%, respectively, to the mean total TEQ concentration. Overall, the mean total TEQ concentration in gull embryos from Hamilton Harbour in 2015 was within the range of mean TEQ concentrations in herring gull eggs from other colonies on the Great Lakes (Figure 3; ranked as means from highest to lowest; ECCC unpublished). Also noteworthy is that the total TEQ concentration in Hamilton Harbour embryos in 2015 fell within the range of total TEQs in embryos at Scotch Bonnet Island in 2013 (205.46 pg TEQ/g; measured as part of a separate study) and Salmon Island in 2015, the two Lake Ontario reference colonies in this study.

Table 3. Concentrations of non-*ortho* PCBs, 2,3,7,8-TCDD, and 2,3,7,8-TCDD toxic equivalents as TEQs (pg/g, wet weight) in embryos of herring gulls from the Hamilton Harbour AOC colony (Neare Island) and the corresponding reference colony (Salmon Island) in 2015. Each sample is a single pooled sample consisting of 15 embryos. TEQs associated with 4 non-*ortho* PCBs, 17 dioxins and furans (PCDD/Fs), and 3 mono-*ortho* PCBs (#105, #118, and #156) and which together comprise total TEQs are also provided.

Colony	**AOC/ Ref**	**Year**	**PCB-77**	**PCB-81**	**PCB-126**	**PCB-169**	**2,3,7,8 – TCDD**	**TEQ – non-*ortho* PCBs**	**TEQ – PCDD/Fs**	**TEQ – mono-*ortho* PCBs**	**Total TEQs**
Neare I.	AOC	2015	163.00	58.75	939.50	141.50	5.77	108.12	8.32	14.16	130.60
Salmon I.	Ref	2015	61.00	56.40	639.00	100.00	7.13	72.69	10.87	6.88	90.44

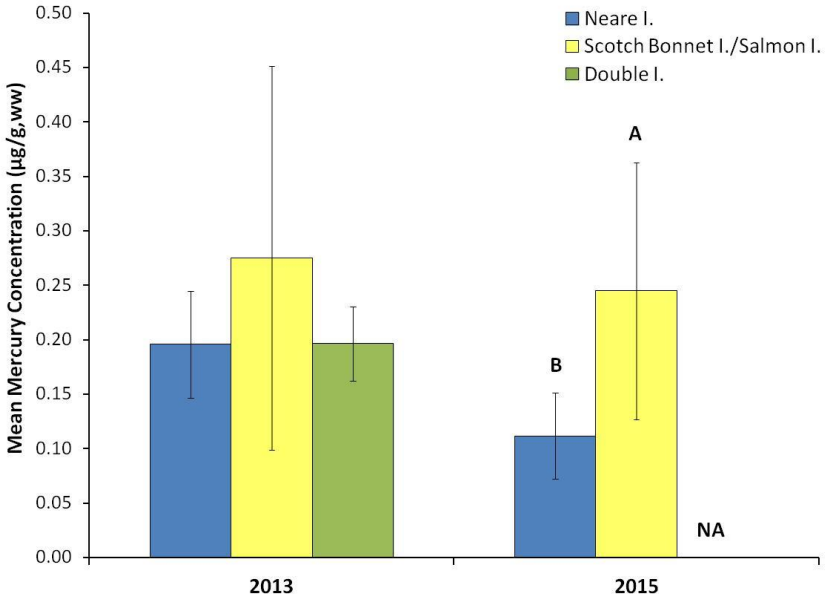
Figure 3. Total TEQ concentrations (pg/g, wet weight) in herring gull embryos (N=1 pooled sample) from the Hamilton Harbour AOC colony (Neare Island, 2015) and corresponding reference colonies, Scotch Bonnet Island (2013) and Salmon Island (2015). Mean total TEQ concentrations (SD) in herring gull eggs from Great Lakes colonies from 2012–2014 are also provided for comparison purposes with the exception of Spanish Harbour where eggs were collected in 2011 and 2012 (N=2 pools). Data for St. Marys River (Ontario) and Thunder Bay are for herring gull embryos collected as eggs and artificially incubated in the lab in 2011 and 2012 (N=6 samples) and 2012 and 2014 (N=4 samples), respectively. The contributions of TEQ concentrations associated with mono-*ortho* PCBs, dioxins and furans, and non-*ortho* PCBs to the total TEQ concentration are shown. Means are arranged in decreasing order from highest to lowest concentrations. Colony locations are associated with the following lakes/rivers: LH=Lake Huron, LO=Lake Ontario, LE=Lake Erie, SLR=St. Lawrence River, NR=Niagara River, LS=Lake Superior, and LM=Lake Michigan.



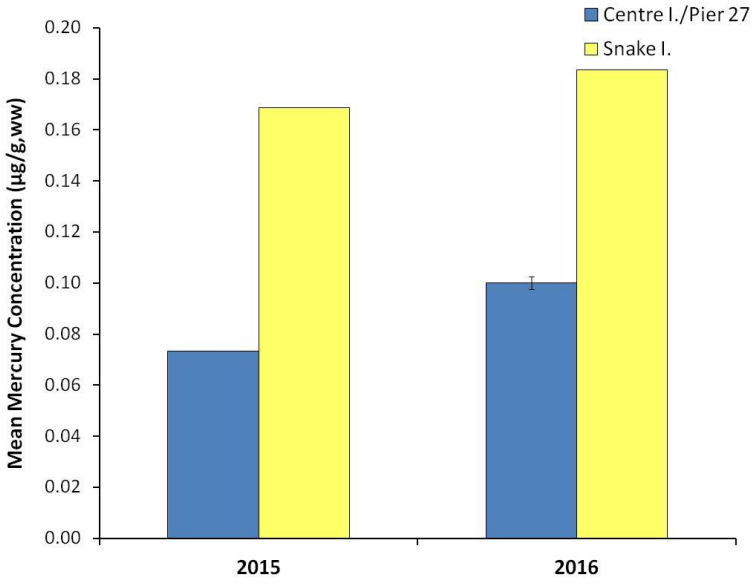
Mean mercury concentrations (SD) in herring gull embryos in 2013 were statistically comparable among study colonies with means ranging from 0.20 (0.05) µg/g wet weight at Neare Island to 0.28 (0.18) µg/g at Scotch Bonnet Island (Figure 4a). In 2015, mean mercury concentrations (SD) were 0.11 (0.04) µg/g at Neare Island and 0.25 (0.12) µg/g at Salmon Island. Mean mercury concentrations were significantly higher in embryos from the Lake Ontario reference colony compared to those from the AOC colony (t_{18}=3.52, p=0.002). The maximum mercury concentration in this study was found in an embryo from Scotch Bonnet Island in 2013 (0.60 µg/g) which was two times higher than the maximum mercury concentration (0.30 µg/g) found in an AOC colony embryo in 2013.

Figure 4. Concentrations of total mercury (µg/g, wet weight) in embryos of herring gulls (a) and eggs of double-crested cormorants (b) from Hamilton Harbour AOC colonies (Neare Island, Centre Island, and Pier 27) and corresponding reference colonies (Scotch Bonnet Island, Double Island, Salmon Island and Snake Island) in 2013, 2015, and 2016. Mean concentrations (SD) in herring gulls are based on 10 or 15 individual embryos per colony. Different uppercase letters show significant differences in mean concentrations among colonies within a study year and are based on statistical analysis of dry weight mercury concentrations. Concentrations in double-crested cormorant eggs are based on analysis of a single pool consisting of 13 eggs at Centre Island in 2015 and at Snake Island in 2015 and 2016 and a mean concentration (SD) at Hamilton Harbour colonies (two pools of eggs from Pier 27 and Centre Island consisting of 12 and 13 eggs, respectively) in 2016. NA indicates that data are not available.

a) Herring gull embryos:

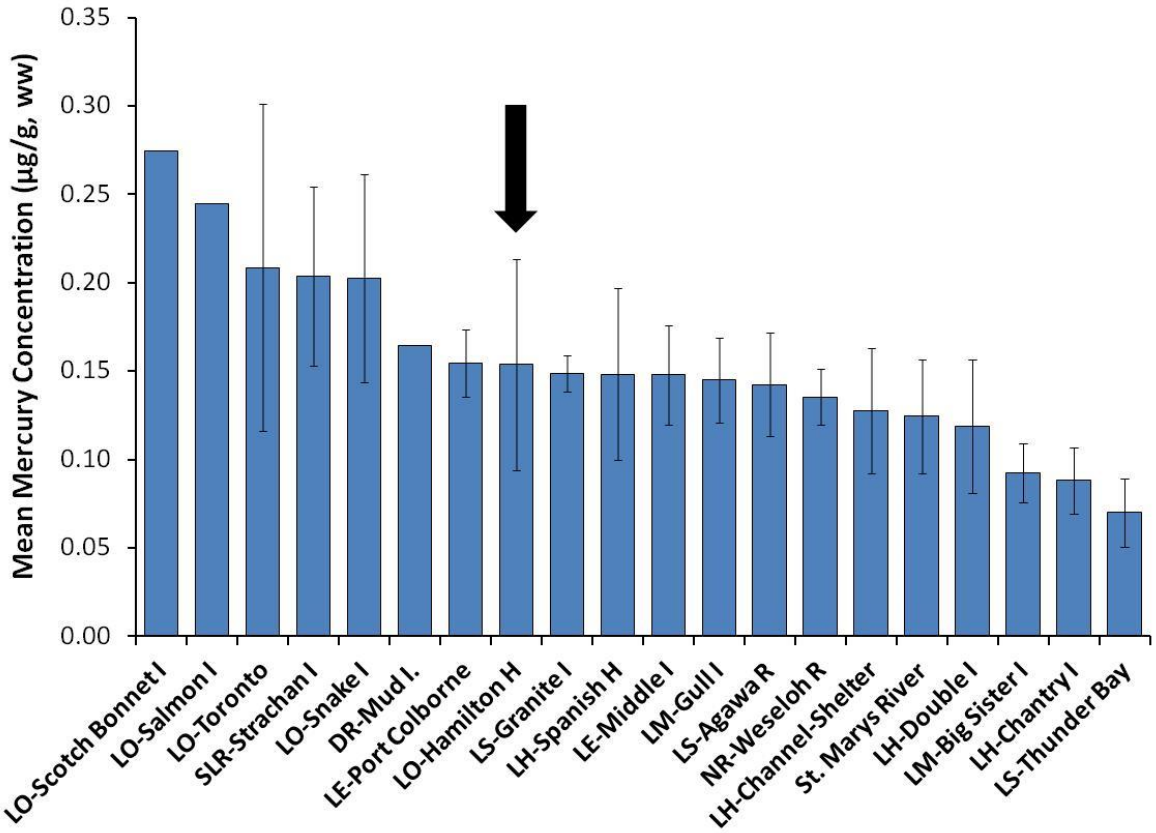


b) Double-crested cormorant eggs:



Overall, mean mercury concentrations in gull embryos from the AOC colony were within the range of mean mercury concentrations reported in herring gull eggs from other Great Lakes colonies from 2013 to 2015 (Figure 5; ranked as means from highest to lowest; ECCC unpublished). In addition, mercury concentrations in embryos from the Hamilton Harbour colony were also well below concentrations found in eggs from the two Lake Ontario reference colonies at Scotch Bonnet Island and Salmon Island which were the highest reported in gulls from Great Lakes colonies. Similar to the TEQ data presented as a broad comparison of Great Lakes trends, results for herring gull embryos collected as eggs and incubated in the lab from the St. Marys River (Ontario) in 2011 and 2012 and Thunder Bay in 2012 and 2014 are included here and data for Spanish Harbour are for herring gull eggs collected in 2011 and 2012.

Figure 5. Mean mercury concentrations (SD, µg/g, wet weight) in herring gull embryos from the Hamilton Harbour AOC colony (Neare Island) in 2013 and 2015 (based on N=2 mean concentrations) and corresponding reference colonies, Scotch Bonnet Island (2013) and Salmon Island (2015). Mean mercury concentrations in herring gull eggs from Great Lakes colonies from 2013–2015 are also provided for comparison purposes with the exception of Spanish Harbour where eggs were collected in 2011 and 2012 (N=2 pools). Data for St. Marys River (Ontario) and Thunder Bay are for herring gull embryos collected as eggs and artificially incubated in the lab in 2011 and 2012 and in 2012 and 2014, respectively (N=4 mean concentrations, both locations). Codes for lakes and rivers associated with colony locations are provided in caption for Figure 3.



Mercury concentrations were also higher in cormorant eggs from the Lake Ontario reference colony at Snake Island compared to Hamilton Harbour AOC colonies in 2015 and 2016 (Figure 4b). The mercury concentration in double-crested cormorant eggs from Centre Island in 2015 was 0.07 µg/g and as a mean concentration (SD) based on two pooled samples at the two AOC colonies, Centre Island and Pier 27, was 0.10 (0.002) µg/g in 2016. Concentrations of mercury in eggs from the Lake Ontario reference colony at Snake Island were nearly two times higher than at the AOC colonies and equal to 0.17 µg/g and 0.18 µg/g in 2013 and 2015, respectively. Comparing burdens between the two colonial waterbird species at AOC colonies, concentrations of mercury in cormorant eggs in 2015 and 2016 were within the range of concentrations in gull embryos in 2013 and 2015.

Temporal Trends of Contaminants in Herring Gulls and Cormorants:

Long term collections and contaminant analysis of herring gull eggs from Hamilton Harbour allow for an assessment of temporal trends in exposure in gulls foraging within the AOC. As part of the Great Lakes Herring Gull Egg Monitoring Program, eggs have been collected annually from selected nesting colonies in Hamilton Harbour since 1981 and analyzed for a suite of contaminants (generally as a single pooled sample; see Hughes *et al.* 2016 for further details). Temporal trends indicate that concentrations of sum PCBs (based on the sum concentration of common PCB congeners), most organochlorines, sum PBDEs, mercury, 2,3,7,8-TCDD, and total TEQs declined significantly in herring gull eggs collected from Hamilton Harbour from the 1980s/1990s (or 2000) to 2016 (Figure 6; range in r^2=0.34–0.79; $p<0.02$). The single exception was for OCS where no significant decline was found in eggs from 1987 to 2016 following high among-year variability in early years. This compound was found at the lowest concentration relative to others. Overall, large decreases in concentrations of compounds, ranging from 51% for OCS to 97% for sum chlordane, were found in gull eggs between the first year and last year of analysis. With respect to the comparability of contaminants between herring gull eggs and gull embryos in this study, concentrations of organochlorines and mercury in pooled gull eggs collected from 2013 to 2015 were generally within the ranges of concentrations for respective compounds in embryos in 2013 and 2015. Two exceptions were OCS and HCB which were found at relatively higher concentrations in eggs compared to embryos; these compounds were also found at the lowest concentrations. Concentrations of 2,3,7,8-TCDD in gull eggs in 2013 and 2014 (when they were last measured) were slightly higher (6.59 pg/g and 7.91 pg/g) compared to the concentration in embryos in 2015 (5.77 pg/g). Total TEQ concentrations in gull eggs in 2013 and 2014 (126.80 pg/g and 146.10 pg/g, respectively) were similar to the concentration in embryos in 2015 (130.60 pg/g). As would be expected, contaminant concentrations in gull embryos in this study are largely comparable to concentrations in fresh gull eggs.

Large decreases in contaminant burdens were also evident in cormorant eggs collected from Pier 27 in 1989 relative to current concentrations reported in eggs collected from Centre Island and Pier 27 in 2016 (shown as a mean for the two pooled samples; Figure 7). Sum PCB burdens in cormorants decreased by 83% from an initial concentration of 9.35 µg/g in eggs in 1989 (analyzed as a single pool) to 1.60 µg/g in eggs from Centre Island & Pier 27 in 2016 (based on sum concentrations of 35–42 PCB congeners over the five years). When the same 25 PCB congeners used to calculate sum PCB concentrations were compared between 1989 and 2016, the percent decrease (83%) remained the same between the two years. Large decreases were also found for seven other organochlorines in cormorant eggs between these two years

Figure 6. Temporal trends in concentrations of 12 contaminants in herring gull eggs collected annually from selected nesting colonies in Hamilton Harbour from the 1980s/1990s (or 2000 for sum PBDEs) to 2016. White open circles represent estimated sum PCB concentrations (see methods in Hughes *et al*. 2016). Concentrations are based on analysis of a single pooled sample of eggs with the exception of 1981 where a mean concentration based on 10 eggs is shown. Concentrations, as wet weights, are in µg/g for all contaminants except TCDD and total TEQs which is in pg/g. Data for total TEQs are based on concentrations of dioxin-like PCBs, furans, and dioxins in years when these three sets of compounds quantified. Exponential curves are provided where temporal declines were significant.

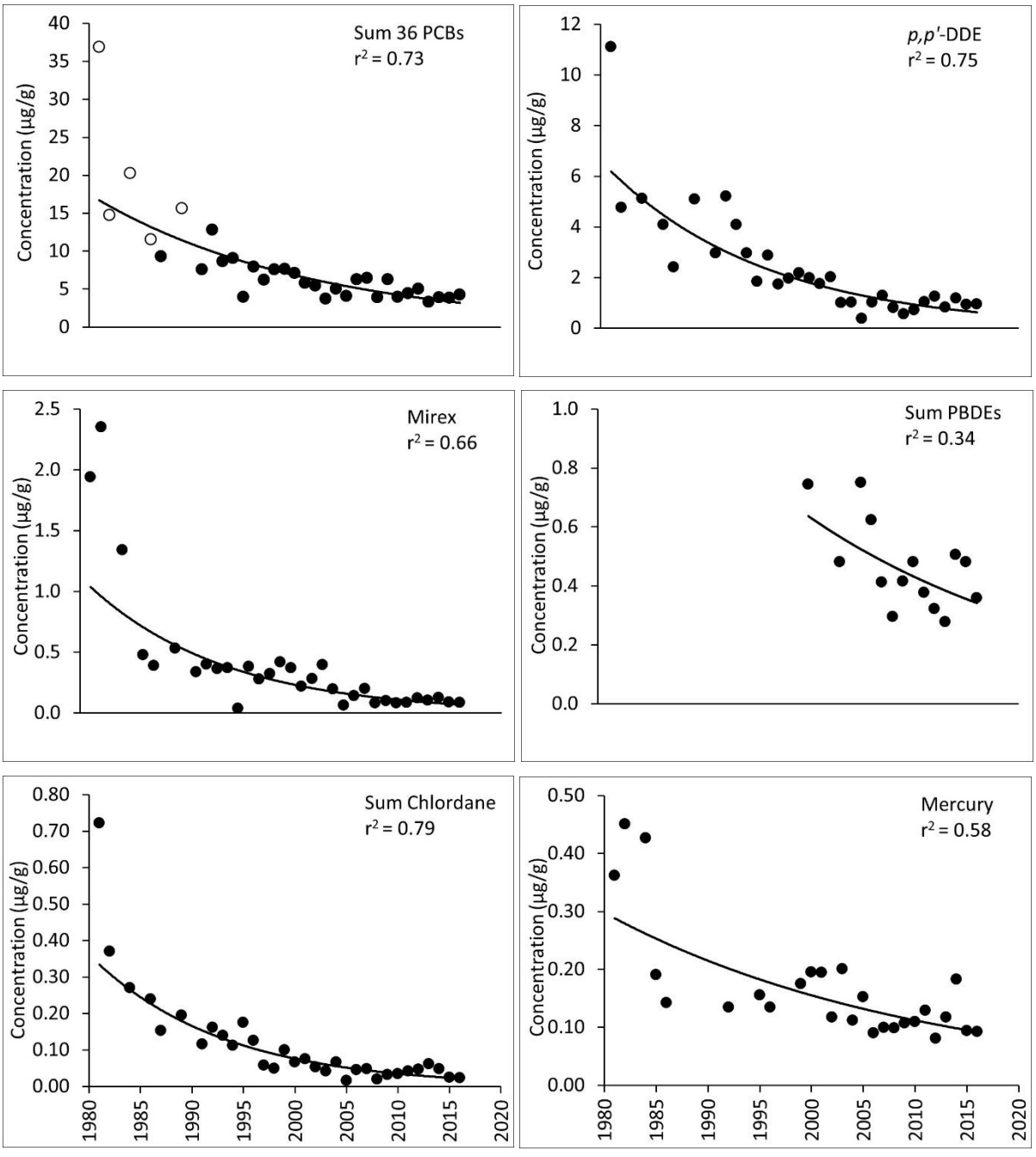


Figure 6 (continued).

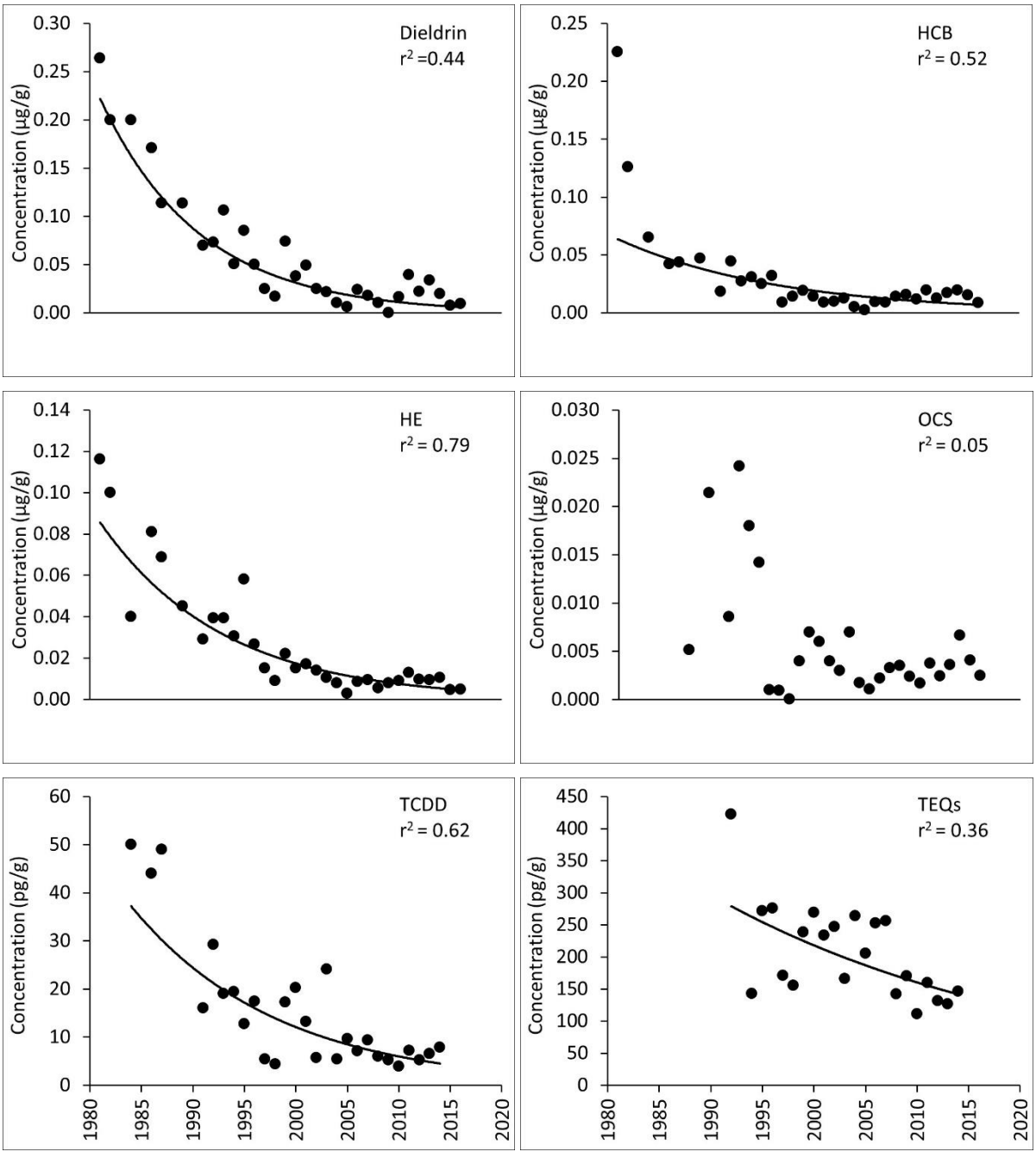
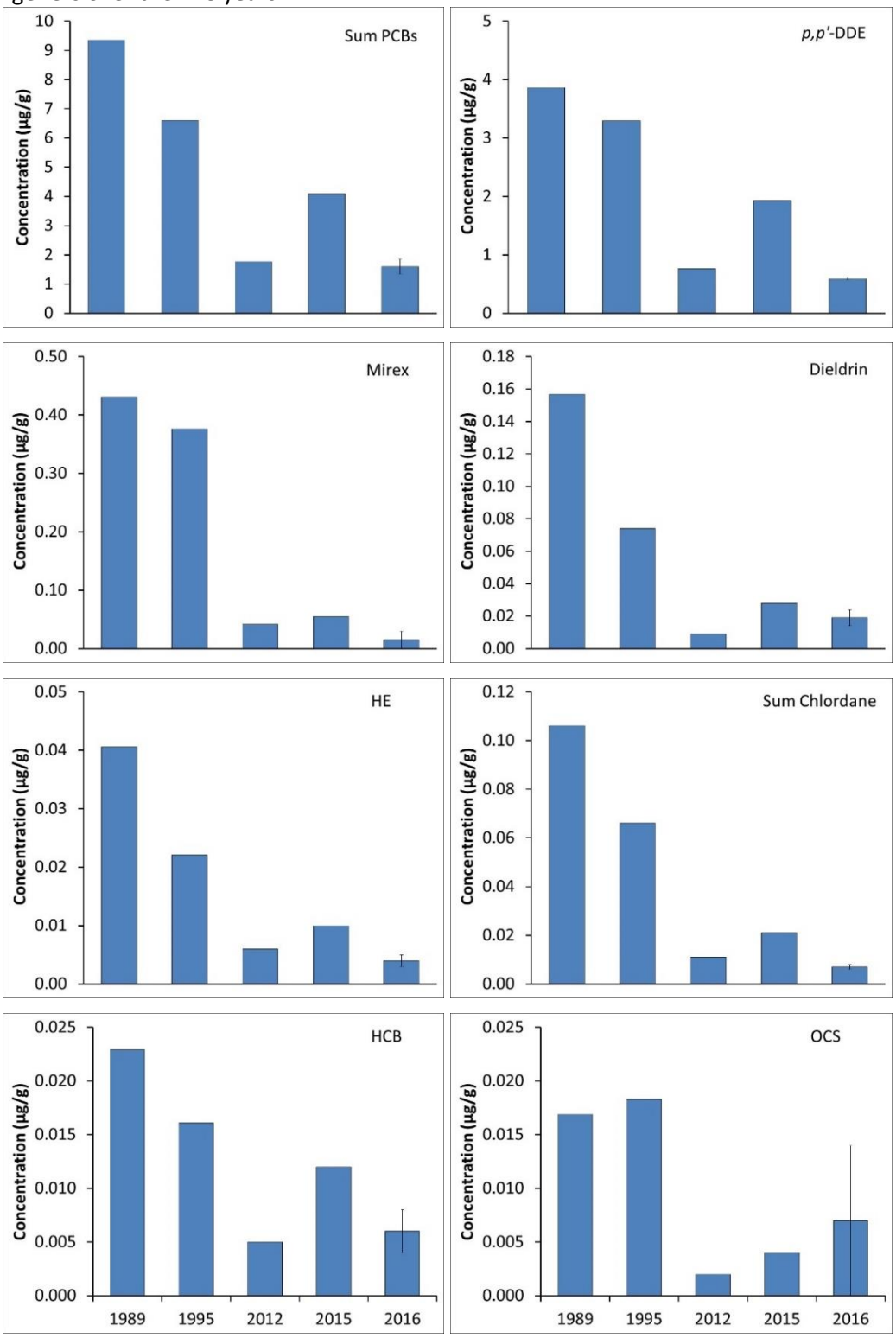


Figure 7. Temporal trends in concentrations (µg/g) of eight organochlorines in double-crested cormorant eggs from Hamilton Harbour AOC colonies from 1989 to 2016. Concentrations, as wet weights, are based on analysis of a single pooled sample of 10–13 eggs. Mean concentrations (SD) are shown for eggs collected from Centre Island and Pier 27 in 2016. Sum PCBs are based on sum concentrations of 35–42 PCB congeners over the five years.



(range=59%–97%). Similar to herring gulls, exposure to organochlorines has decreased in cormorants nesting in the AOC since the 1980s.

Stable Isotopes:

Significant spatial differences for mean $\delta^{15}N$ values in herring gull embryos were found among study sites in 2013 ($F_{2,27}$=49.50, p<0.00001) and 2015 (t_{28}=7.69, p<0.00001; Table 4a). As an indicator of trophic position, mean $\delta^{15}N$ values were significantly higher in gulls from the Hamilton Harbour AOC colony compared to one or both of the reference colonies in both study years. In addition, gull embryos from the AOC colony in 2013 had a mean $\delta^{13}C$ value that was also significantly more depleted (i.e., more negative) than the mean value in embryos from the Lake Huron Double Island reference colony but that was not significantly different than the mean value at the Lake Ontario reference colony ($F_{2,27}$=3.90, p=0.03). In 2015, mean $\delta^{13}C$ values were not significantly different between the AOC colony and the Lake Ontario reference colony. No significant correlation was found between $\delta^{15}N$ and $\delta^{13}C$ values when herring gull embryos from the AOC colony were grouped together in the two study years.

Table 4. Values for $\delta^{15}N$ and $\delta^{13}C$ in embryos of herring gulls (a) and eggs of double-crested cormorants from Hamilton Harbour AOC colonies (Neare Island, Centre Island, and Pier 27) and corresponding reference colonies (Scotch Bonnet Island, Double Island, and Salmon Island) in 2013, 2015, and 2016. Mean (SD) values in herring gulls are based on 10 or 15 embryos per colony. Different uppercase letters show significant differences in mean values among gull colonies within a study year. Values in double-crested cormorant eggs are based on analysis of a single pool consisting of 13 eggs at Centre Island in 2015 and at Snake Island in 2016 and a mean concentration (SD) at Hamilton Harbour colonies (two pools of eggs from Pier 27 and Centre Island consisting of 12 and 13 eggs, respectively) in 2016. NA indicates that data are not available.

a) Herring gull embryos:

Colony	AOC/Ref	$\delta^{15}N$		$\delta^{13}C$	
		2013	2015	2013	2015
Neare I.	AOC	15.76 (0.79) **A**	15.52 (1.17) **A**	-22.02 (0.38) **B**	-21.06 (0.67)
Scotch Bonnet I./ Salmon I.	Ref	13.77 (2.34) **B**	11.53 (1.63) **B**	-21.56 (1.30) **AB**	-20.85 (1.55)
Double I.	Ref	9.46 (0.99) **C**	NA	-21.00 (0.44) **A**	NA

b) Double-crested cormorant eggs:

Colony	AOC/Ref	$\delta^{15}N$		$\delta^{13}C$	
		2015	2016	2015	2016
Centre I./Pier 27	AOC	17.66	18.13 (0.08)	-24.27	-23.56 (0.30)
Snake I.	Ref	16.29	16.09	-21.27	-20.71

Relative to gulls, similar stable isotope patterns were found in cormorant eggs between study colonies in 2015 and 2016 (Table 4b). Eggs from the AOC colonies had relatively higher $\delta^{15}N$ values and more depleted $\delta^{13}C$ values compared to cormorant eggs from the Snake Island reference colony. Comparing isotopic signatures between the two colonial waterbird species at AOC colonies, $\delta^{15}N$ values were

relatively higher and $\delta^{13}C$ values were relatively lower in cormorant eggs compared to values in herring gull embryos in the two study years.

B) Field Study

Egg Size Parameters:

To assess potential food stress in birds, total clutch volume and intraclutch variation in egg size were examined in 3-egg clutches in 2013, 2015, and 2016. Since no significant difference in total clutch size or intraclutch variation was found in 2016 between the two AOC colonies at Neare Island and Pier 27, egg data were combined for these two colonies. Mean total clutch volume (SD) in herring gull eggs from Hamilton Harbour AOC colonies ranged from 256.9 (24.1) cm^3 at Neare Island/Pier 27 in 2016 to 271.7 (18.3) cm^3 at Neare Island in 2013 (Figure 8a). Total clutch volume in gull eggs from Lake Ontario and Lake Huron reference colonies ranged from 235.3 (19.4) cm^3 at Double Island in 2016 to 271.3 (18.3) cm^3 at Scotch Bonnet Island in 2013. Mean total clutch volumes varied significantly among colonies with similar spatial patterns evident in the three study years. Mean total clutch volumes at Hamilton Harbour AOC colonies were significantly higher than clutch volumes at the Lake Huron reference colony but were statistically similar to total clutch volumes at the Lake Ontario reference colonies in 2013 ($F_{2,66}$=16.42, p<0.00001), 2015 ($F_{2,81}$=7.29, p=0.001), and 2016 ($F_{2,55}$=13.22, p=0.00002).

Mean intraclutch variation (SD) in egg size at AOC colonies ranged from 5.0 (2.9)% at Neare Island in 2013 to 6.3 (3.5)% at Neare Island/Pier 27 in 2016 (Figure 8b). Mean intraclutch variation in gull eggs from Lake Ontario and Lake Huron reference colonies ranged from 6.3 (1.7)% at Scotch Bonnet Island in 2013 to 11.5 (4.4)% at Double Island in 2013. Overall, mean intraclutch variation in clutches from Hamilton Harbour colonies was significantly lower than clutches from Lake Huron reference colony but was statistically similar to variation in egg size of clutches from Lake Ontario reference colonies in 2013 (F_{266}=25.94, p<0.00001) and 2015 ($F_{2,81}$=8.93, p=0.0003). No significant difference was found for this parameter among study sites in 2016.

Productivity & Prevalence of Deformities in Wild Colonial Waterbird Populations:

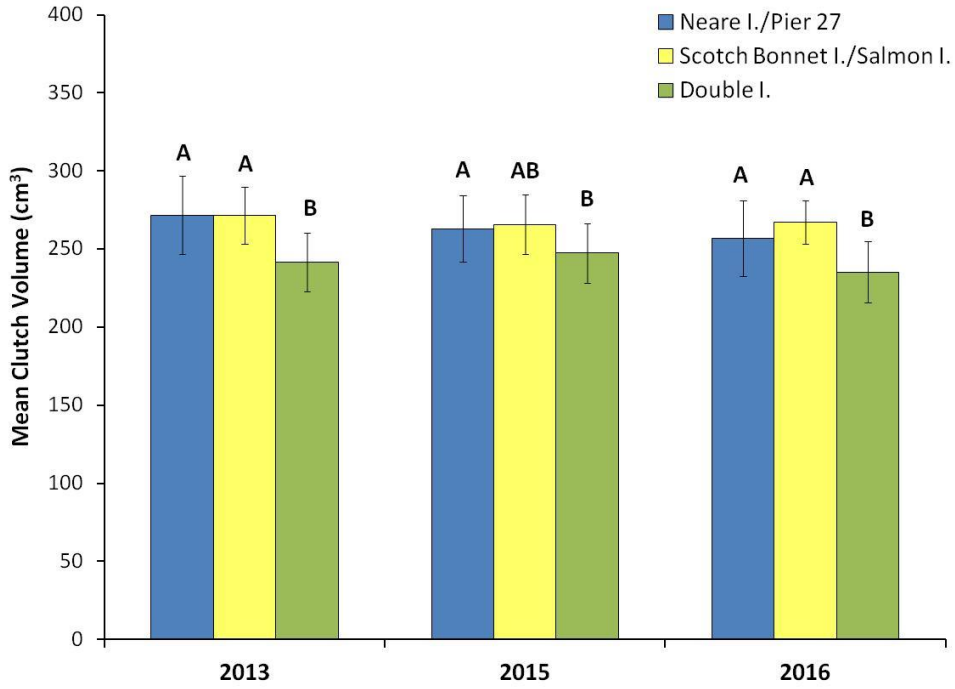
In 2013, herring gull productivity, defined as the number of 21-day-old chicks produced per nest, was equal to 2.4 chicks per nest at the Hamilton Harbour AOC colony (N=10 enclosed nests) and 2.3 chicks per nest at Double Island (N=12 nests; Figure 9). Productivity was relatively lower at the AOC colony and two reference colonies in 2015 and equal to 1.7 chicks per nest at Neare Island (N=10 enclosed nests), 1.5 chicks per nest at Salmon Island (N=12 enclosed nests), and 1.7 chicks per nest at Double Island (N=10 enclosed nests). Overall, productivity estimates at the AOC colony in the two study years exceeded the range of productivity levels required to maintain a stable population (0.8–1.4 chicks per nest; Kadlec and Drury 1968).

In 2013 and 2015, no morphological deformities were found in herring gull chicks from the AOC colony at Neare Island or the reference colonies (based on 17–41 chicks examined; Table 5). This count included 14 chicks from outside of the enclosures at Double Island in 2013.

In 2013, deformity surveys of cormorant chicks at Centre Island, Pier 27, and Mohawk Island revealed a low overall prevalence of chick deformities at the three colonies (Table 6). In total, one deformed chick

Figure 8. Total clutch volume (a) and intraclutch variation (b) in 3-egg clutches of herring gulls from Hamilton Harbour AOC colonies (Neare Island and Pier 27) and corresponding reference colonies (Scotch Bonnet Island, Double Island, and Salmon Island) in 2013, 2015, and 2016. Numbers of 3-egg clutches ranged from 12–42 at each colony. Different uppercase letters show significant differences in mean estimates within a study year.

a) Mean total clutch volume (SD):



b) Mean intraclutch variation (SD) in egg size:

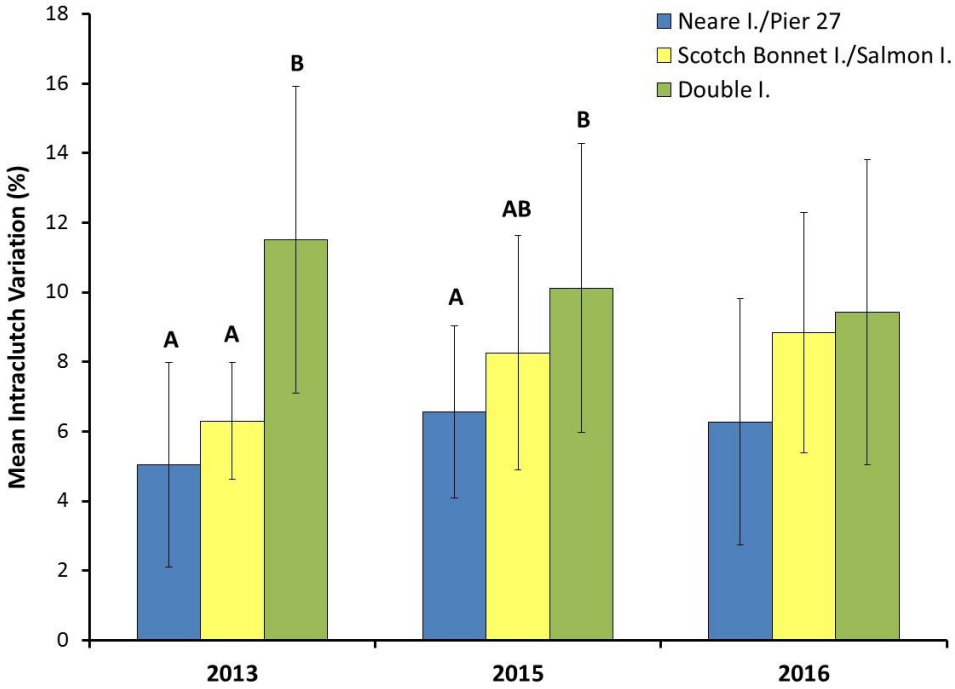


Figure 9. Herring gull productivity, calculated as the number of 21-day-old chicks produced per nest, at the Hamilton Harbour AOC colony (Neare Island) and reference colonies (Salmon Island and Double Island) in 2013 and 2015. The solid line indicates the minimum productivity level of 0.8 chicks per 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.

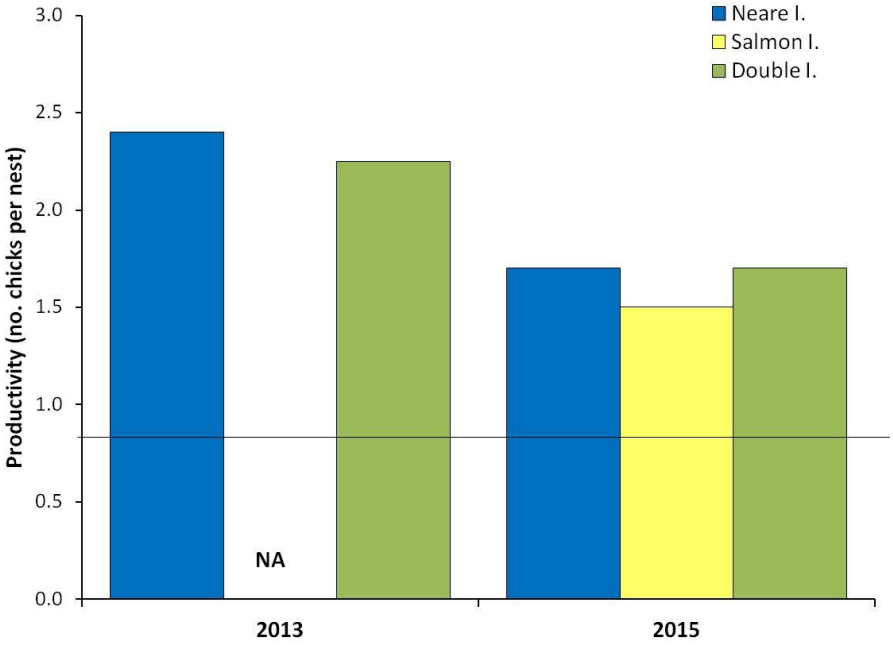


Table 5. Prevalence of morphological deformities (%) in herring gull chicks examined in enclosed nests from the Hamilton Harbour AOC colony (Neare Island) and reference colonies (Salmon Island and Double Island) in 2013 and 2015.

Colony	Year	No. Chicks Examined	% Deformities
Neare I.	2013	24	0%
	2015	17	0%
Salmon I.	2015	18	0%
Double I.	2013	41*	0%
	2015	17	0%

* includes 14 chicks from non-enclosed nests

Table 6. Prevalence of morphological deformities (%) in double-crested cormorant chicks from two Hamilton Harbour AOC colonies (Centre Island and Pier 27) and the reference colony (Mohawk Island, Lake Erie) in 2013. Lower and upper 95% confidence intervals are also shown.

Colony	AOC/Ref	No. Chicks Examined	No. Chicks with Deformity	% Deformities	Lower 95% CI	Upper 95% CI
Centre I.	AOC	1,618	1	0.062%	0.0016%	0.34%
Pier 27	AOC	727	0	0%	0%	0.51%
Overall	**AOC**	**2,345**	**1**	**0.043%**	**0.0011%**	**0.24%**
Mohawk I.	**Ref**	**1,683**	**0**	**0%**	**0%**	**0.22%**

with a crossed bill was found at Centre Island where 1,618 chicks were examined (0.062%) and no deformities were found in 727 chicks examined at Pier 27 (0%). No deformities were found in 1,683 chicks examined at the Mohawk Island reference colony (0%). The prevalence of cormorant chicks with deformities was not significantly different between each of the two AOC colonies and the reference colony.

Corticosterone in Feathers of Herring Gull Chicks:

Mean corticosterone concentrations (SD) in feathers of herring gull chicks from the AOC colony at Neare Island ranged from 1.7 (2.9) pg/mm in 2015 to 2.6 (0.4) pg/mm in 2016 (Figure 10). Mean corticosterone concentrations (SD) in chick feathers from the reference colonies ranged from 1.4 (0.8) pg/mm at Salmon Island in 2015 to 3.3 (0.9) pg/mm at Double Island in 2016. Corticosterone concentrations in feathers were not significantly different among colonies in any of the three study years.

Thyroxine in Plasma of Herring Gull Chicks:

Mean thyroxine concentrations (SD) in plasma of herring gull chicks from the AOC colony at Neare Island ranged from 0.78 (0.28) ng/dl in 2015 to 1.28 (0.27) ng/dl in 2013 (Figure 11). Mean thyroxine concentrations (SD) in plasma of chicks from the reference colonies were comparatively lower and ranged from 0.21 (0.10) ng/dl at Double Island in 2016 to 1.04 (0.41) ng/dl at Double Island in 2013 in the three study years. Mean thyroxine concentrations were significantly higher in plasma of chicks from the AOC colony compared to the two reference colonies in 2016 (Kruskal Wallis H=13.20, p=0.001) while thyroxine concentrations were not significantly different among sites in 2013 and 2015.

Immune Response in Herring Gull Chicks:

Estimates of the PHA stimulation index in chicks from the AOC colony at Neare Island, expressed as means (SD), were 0.14 (0.17) mm in 2015 and 0.23 (0.17) mm in 2016 (Figure 12). Estimates of the index in chicks from the reference colonies, as means (SD), ranged from 0.13 (0.16) mm at Salmon Island in 2016 to 0.36 (0.12) mm at Double Island in 2015. In 2015, the stimulation index was significantly lower in chicks from the AOC colony compared to the Double Island reference colony but was not significantly different from the Lake Ontario reference colony ($F_{2,41}$=6.48, p=0.004). This pattern was not found in 2016 when no significant difference in PHA response in herring gull chicks was found among study sites.

Figure 10. Mean corticosterone concentrations (SD), expressed as picograms of corticosterone per millimetre of feather, in herring gull chicks from the Hamilton Harbour AOC colony (Neare Island) and reference colonies (Salmon Island and Double Island) in 2013, 2015, and 2016. Numbers of chicks sampled ranged from 7–15 per site NA indicates that data are not available.

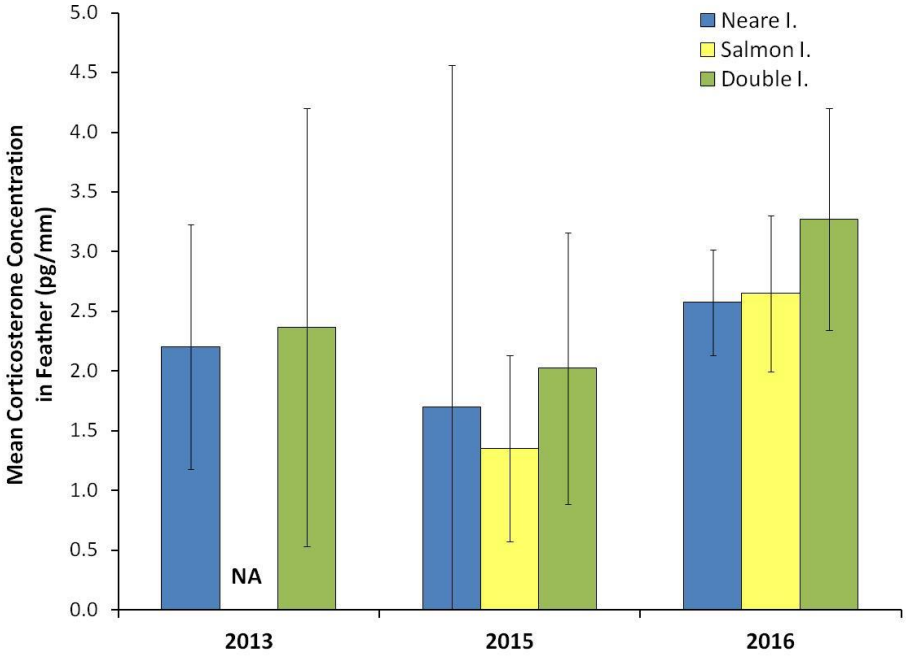


Figure 11. Mean thyroxine concentrations (SD) in plasma of herring gull chicks from the Hamilton Harbour AOC colony (Neare Island) and reference colonies (Salmon Island and Double Island) in 2013, 2015, and 2016 (ng/dl). Numbers of chicks sampled ranged from 7–15 per site. Different uppercase letters show significant differences in mean concentrations within a study year. NA indicates that data are not available.

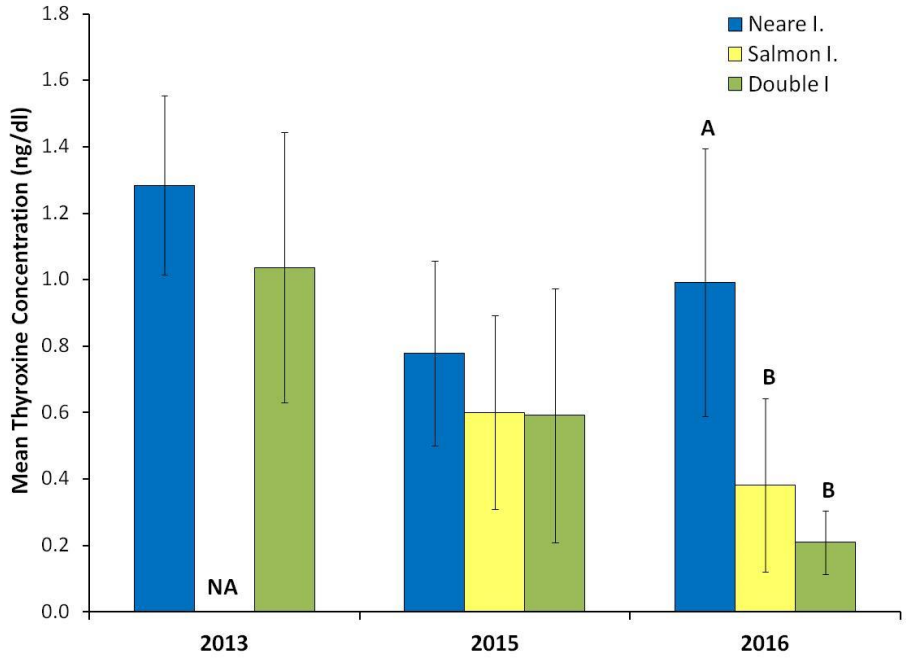
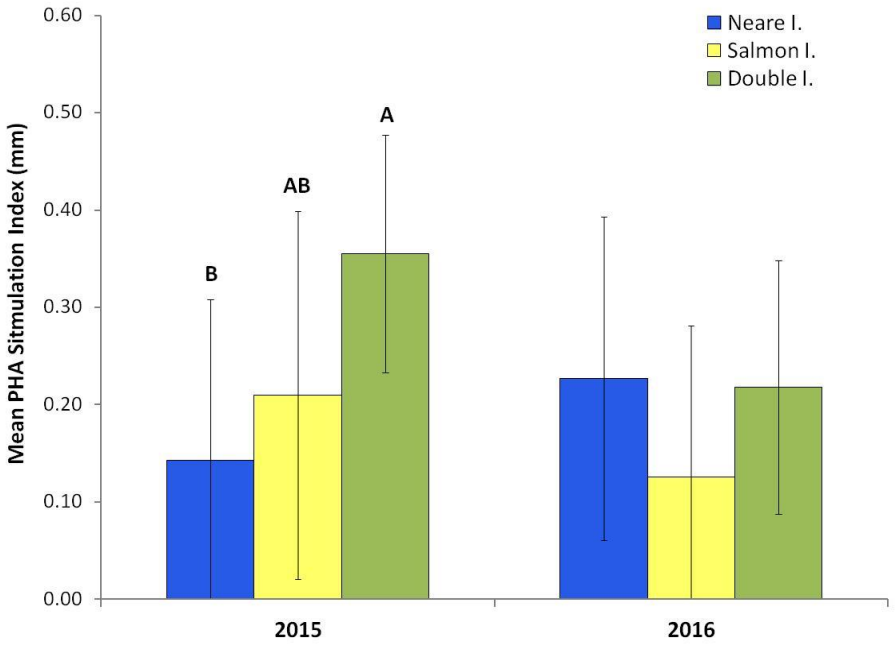


Figure 12. Mean PHA stimulation index (SD), expressed in millimetres, in herring gull chicks from the Hamilton Harbour AOC colony (Neare Island) and reference colonies (Salmon Island and Double Island) in 2015 and 2016. Numbers of chicks sampled ranged from 7–15 per site. Different uppercase letters show significant differences in the mean PHA stimulation index within a study year.



DISCUSSION

Using a multi-tiered lab and field study approach, assessments of reproduction and deformities in herring gulls nesting in the Hamilton Harbour AOC were conducted in three study years. Artificial incubation of freshly-laid eggs under controlled conditions in the laboratory were valuable for assessing the importance of intrinsic factors such as contaminants that may induce early embryonic mortality or result in developmental abnormalities at this critical life stage. In this study, embryonic viability in artificially incubated herring gull eggs from the AOC colony was high at 100% in both study years. This is also consistent with the results of an earlier study in which egg viability in herring gulls from Neare Island, based on field assessments, was also high. Egg viability in 3-egg clutches from the AOC colony, as means (SD), was equal to 96.4 (10.4)% and 97.5 (8.8)% in 2003 and 2004, respectively, and were among the highest reported at Great Lakes colonies from 2000–2006 (Hughes *et al.* 2010). In the current study, no embryonic deformities were evident in incubated eggs from the AOC colony in the two study years.

Reproduction based on productivity values for herring gulls nesting on Neare Island in the Hamilton Harbour AOC was considered good with productivity equal to 2.4 chicks per nest and 1.7 chicks per nest in 2013 and 2015, respectively. These values were above productivity levels required to maintain a stable herring gull population (Kadlec and Drury 1968). Current estimates of productivity in the AOC were also well above productivity estimates for Lake Ontario gull colonies in the early-mid 1970s when productivity was extremely low (fewer than 0.2 chicks per nest; Gilbertson 1974b; Gilman *et al.* 1977) and were more comparable to estimates in the early 1980s by which time productivity at Lake Ontario colonies had improved considerably (range=1.2–2.1 chicks per nest; Environment Canada *et al.* 1991).

No morphological deformities were found in juvenile herring gull chicks from the AOC colony or reference colonies in this study. While a single deformed cormorant chick was found at Centre Island, the prevalence of deformities in cormorant chicks is considered to be low overall at the two AOC colonies in 2013 (0.043% or 4.3 per 10,000 chicks) and on par with deformity frequencies reported for cormorant chicks in earlier Lake Ontario surveys. Two large-scale surveys of bill deformities in cormorant chicks were conducted on the Great Lakes by the Canadian Wildlife Service in 1979–1987 and 1988–1996 (Fox *et al.* 1991; Ryckman *et al.* 1998). Based on the results of these surveys, the prevalence of bill defects in cormorant chicks from Lake Ontario was 3.5 per 10,000 chicks at two colonies (in the eastern basin) in 1979–1987 and 2.8 per 10,000 chicks at seven colonies (that included a colony in Hamilton Harbour) in 1988–1996. These estimates were based on multiple visits to colonies over several years which differed from the current survey that was conducted on one visit in a single year. In contrast, deformity frequencies in the current study were more than ten times lower than those in cormorant surveys conducted from 1979–1987 and 1988–1990 in Green Bay, Lake Michigan, at a relatively more contaminated site, where deformity frequencies ranged from 52.1 to 86.0 per 10,000 chicks (Fox *et al.* 1991; Larson *et al.* 1996). Deformity frequencies in cormorant chicks from the two AOC colonies in this study were also not significantly different from that found in chicks from the Mohawk Island reference colony in 2013.

Based on the contaminant burdens reported in herring gull embryos and double-crested cormorant eggs in 2013, 2015 and 2016, concentrations of PCBs, *p*,*p*'-DDE, and PBDEs were not sufficiently elevated to adversely impact the reproductive success of these species foraging in the Hamilton Harbour AOC. In a broad literature review of PCB effects in birds, Hoffman *et al.* (1996) concluded that sum PCB concentrations in the range of 8 to 25 µg/g in eggs were associated with decreased hatching success for terns and cormorants. Sum PCB concentrations in embryos and eggs of both species were below the 8 µg/g threshold. Similarly, concentrations of *p*,*p*'-DDE in embryos and eggs were well below threshold levels associated with significant effects on reproductive success as reported in black-crowned night-herons (*Nycticorax nycticorax;* 8 µg/g; Henny *et al.* 1984) and cormorants (10 µg/g; Pearce *et al.* 1979). Sum PBDE concentrations in gulls and cormorants from AOC colonies were below the lowest-observed effect level on pipping and hatching success in American kestrels (*Falco sparverius*) equal to 1.8 µg/g in eggs (McKernan *et al.* 2009).

Significantly higher concentrations of some persistent organic pollutants, notably PCBs and *p*,*p*'-DDE, were found in herring gull embryos from the AOC colony compared to the Lake Huron reference colony in 2013 and the Lake Ontario reference colony in 2015 (Table 2). Similar spatial patterns for PCBs have been found in surface sediment in the main basin of the Harbour and young of year fish from the Harbour relative to other non-AOC areas in Lake Ontario (Labencki 2008). Snapping turtle eggs collected from locations within the AOC at Grindstone Creek and Cootes Paradise had the highest concentrations of PCBs, several organochlorine pesticides, and PBDEs compared to other Great Lakes AOCs within the lower Great Lakes basin from 2001–2004 (de Solla *et al.* 2007). Several factors have contributed to relatively higher levels of contamination in Hamilton Harbour compared to other Lake Ontario areas. The Harbour is surrounded by several major industries and large urban centres with effluent from three (formerly four) wastewater treatment plants that discharge into the Harbour. In addition, three main tributaries drain the entire watershed (approximately 500 km^2 in size) and flow into the Harbour. The

physical attributes of the Harbour, as a shallow embayment, connected by a narrow shipping channel limits the extent of water circulation with the rest of Lake Ontario. While legislation and abatement programs have significantly reduced loadings of pollutants into the Harbour, loadings from historically contaminated sediment continue to provide ongoing sources that are available to biota. Additionally, there are localized areas of historic PCB contamination within the Harbour including at Windermere Basin and Windermere Arm (Labencki 2008). The availability of PCBs (and other compounds including metals) to biota was effectively demonstrated in 1986 when flightless domestic ducks released into Windermere Basin rapidly accumulated contaminants while feeding in this shallow wetland over a 41-day period (Weseloh *et al.* 1994). Despite observed spatial differences in concentrations of some organochlorines found in embryos between AOC and reference colonies, as described previously, concentrations of these compounds in herring gull embryos from the AOC colony would not be expected to adversely impact reproduction or development of gulls.

In general, organochlorine burdens were higher in gull embryos from Scotch Bonnet Island, the Lake Ontario reference colony in 2013, than burdens reported in embryos from the Salmon Island Lake Ontario reference colony in 2015 (Table 2). Furthermore, burdens in gulls from this colony tended to be more similar to concentrations at the Hamilton Harbour AOC colony. This pattern was also found for PCBs and *p*,*p*'-DDE in liver of adult gulls from Scotch Bonnet Island and Hamilton Harbour colonies in 1991 (Fox *et al.* 1998). This finding may be attributed to the location of the island situated nearly five kilometres offshore, downstream from several major point sources of pollution on the Great Lakes, and on Lake Ontario, a lake that receives the inflow from the rest of the Great Lakes basin. Concentrations of total TEQs were also relatively higher in embryos from Scotch Bonnet Island in 2013 compared to the AOC colony in 2015 (Figure 3). Despite being removed from localized sources of pollutants, the cumulative effects of upstream inputs at Scotch Bonnet Island, an offshore location, is an important contributor to contamination in this area of the Great Lakes.

The total TEQ concentration in gull embryos from the AOC colony in 2015 was not notably elevated and was within the range of TEQ concentrations in herring gull eggs from other Great Lakes colonies from 2012–2014 (Figure 3). Concentrations of dioxin-like PCBs, i.e., non-*ortho* PCBs and mono-*ortho* PCBs, in embryos from the AOC colony contributed more to overall toxicity relative to TCDD and dioxins and furans (concentrations not shown) compared to the reference colony (Table 3). With respect to concentrations associated with toxicity, 2,3,7,8-TCDD (<6 pg/g) and total TEQs (<130 pg TEQ/g) in herring gull embryos from the AOC colony were below concentrations associated with effects on reproduction in avian species. Median concentrations of 2,3,7,8-TCDD (37 pg/g) and total TEQs (2175 pg TEQ/g) in eggs of Forster's tern (*Sterna forsteri*) from Lake Michigan were associated with a significant reduction in hatching success (TEQs based on concentrations of 2,3,7,8-TCDD and dioxin-like PCBs only; Kubiak *et al.* 1989). No effect on tern hatching success was observed at relatively lower median concentrations of 2,3,7,8-TCDD (8 pg/g) and total TEQs (201 pg TEQ/g) in eggs from the reference colony in 1983 (Kubiak *et al.* 1989). The total TEQ concentration in gull embryos was also well below 1200–1300 pg TEQ/g at which high frequencies (6–10%) of embryonic deformities were found in cormorants at two Lake Michigan colonies in 1988 (Yamashita *et al.* 1993). The concentration of 2,3,7,8-TCDD was approximately three orders of magnitude below the lethal dose associated with 50% embryonic mortality in cormorants (4000 pg/g based on egg injection; Powell *et al.* 1998) and was below

concentrations associated with decreased embryonic growth and edema in herons (150–250 pg/g; Hoffman *et al.* 1996). As effectively demonstrated in the artificial incubation component of this study, these compounds were not sufficiently elevated to impact embryonic viability or deformities in gulls from the AOC colony in 2013 and 2015.

Exposure to high concentrations of mercury can have significant impacts on reproductive success in birds and result in teratogenic effects in avian embryos, as demonstrated in egg injection studies with methylmercury in the laboratory (Fimreite 1974; Hill *et al.* 2008; Heinz *et al.* 2011). Overall, mercury concentrations in all AOC embryos were below the predicted threshold of 0.6 µg/g (wet weight) in eggs set to be protective against adverse reproductive effects for 95% of non-marine avian species (Shore *et al.* 2011) and is consistent with high embryonic viability and normal embryonic development of artificially incubated eggs in the lab. The highest mercury concentrations were found in 2013 in an embryo from Scotch Bonnet Island that approached the threshold concentration and in an embryo from the AOC colony that was approximately one-half of the threshold concentration. Mercury burdens in gull embryos were not elevated at the AOC colony and were comparable to or lower than mean concentrations in embryos from the reference colonies in the two study years (Figure 4a). Similarly, mercury burdens were lower in pooled cormorant eggs from AOC colonies compared to the Lake Ontario reference colonies in 2015 and 2016 (Figure 4b). Mean mercury concentrations in AOC embryos in 2013 and 2015 were within the range of mean concentrations at other Great Lakes colonies where gull eggs were collected from 2013–2015 (Figure 5). Overall, mercury concentrations were low and it is unlikely that mercury concentrations would impact reproduction of breeding colonial waterbirds in the Hamilton Harbour AOC.

Large declines in concentrations of sum PCBs and other organochlorines, 2,3,7,8-TCDD and mercury in gull eggs since the 1980s indicate that exposure to these compounds has decreased in herring gulls foraging in the AOC (Figure 7). Weseloh *et al.* (2011) found a significant decline in mercury in gull eggs from Hamilton Harbour from 1981–2009 when changes in gull diet, as inferred from stable nitrogen isotope analysis, were also taken into account. This is an important consideration since temporal changes in diet in this top predator can influence the interpretation of temporal changes in egg burdens and extent of contaminant availability. Exposure to organochlorines also decreased in cormorants foraging in the AOC based on similar large decreases in concentrations of organochlorines in eggs between 1989 and 2016 (Figure 6). These results are consistent with long-term temporal patterns for PCBs and other compounds reported in snapping turtles, several fish species, and suspended sediment in the Harbour (Burniston *et al.* 2016; Hughes *et al.* 2016; Neff *et al.* 2016). As described above, current contaminant burdens are not sufficiently elevated to impact reproduction or development in two colonial waterbird species breeding in the AOC. External stressors such as interspecific competition and habitat availability can impact a bird's ability to reproduce successfully and these have been identified as important factors influencing distributions of colonial waterbird populations in the AOC (Zanchetta *et al.* 2016). Ongoing monitoring and implementation of management techniques as well as several habitat creation projects conducted over four decades have attempted to curb their effects.

Stable isotopes of nitrogen and carbon are used to provide information on trophic position and carbon source in the food web, respectively (Hobson 1999). Significantly higher $\delta^{15}N$ values were found in gull

embryos at the AOC colony compared to the reference colonies in 2013 and 2015. This suggests that gulls occupied a relatively higher trophic level at the AOC colony compared to gulls from reference colonies. Specifically, gulls at this colony may have fed more on fish (or larger fish) compared to gulls from reference colonies, which fed at a relatively lower trophic level and on a diet that may have included terrestrial food sources such as small mammals, refuse and plant material (Fox *et al.* 1990). Differences in trophic levels between colonies may have contributed to higher concentrations of some organochlorines reported in gull embryos from the AOC colony compared to reference colonies. Based on the isotopic $\delta^{13}C$ signatures, gulls from the AOC colony may have fed more on aquatic-based prey types (with more depleted $\delta^{13}C$ signatures) compared to gulls from the reference colonies which fed more on terrestrial-based prey types (with more enriched $\delta^{13}C$ signatures). This is consistent with the spatial pattern evident for $\delta^{15}N$ in gulls. Noteworthy is that anthropogenic sources of nitrogen such as effluent from wastewater treatment plants can also influence stable nitrogen isotope signatures (Wayland and Hobson 2001) making it difficult to draw conclusions on $\delta^{15}N$ patterns in eggs from the Harbour versus other Lake Ontario locations without further study.

Determinations of total clutch volume and intraclutch variation in 3-egg clutches are informative for evaluating potential food stress during the egg production period. High total clutch volumes and low intraclutch variation in clutches from AOC gull colonies compared to reference colonies in all three study years suggest that food availability was likely not limited for laying females. Total clutch volumes in this study were also comparable to total clutch volumes measured in gull eggs from Neare Island in 2004 (mean=259.7 cm^3, based on 30 clutches; Hughes *et al.* 2010) while intraclutch variation in egg size was less favourable and slightly higher in that study (mean=8.7%). Evidence based on these parameters suggests that food stress is not an issue during the egg production period.

Three additional endpoints relating to growth, development, and immune function of chicks were also measured in this study. Corticosterone deposited in growing feathers provides important insight into the physiology of stress during the period of feather growth (Bortolotti *et al.* 2009). Comparable corticosterone concentrations were found in feathers of herring gull chicks between the AOC colony and the reference colonies in three study years. These results are consistent with those in 2004 when chicks from Neare Island were given a standardized stress challenge and no significant difference in stress response was found between gulls from the AOC and reference colonies (Hughes *et al.* 2010). Thyroxine concentrations were not depressed in chicks from the AOC (and were generally higher) compared to chicks from reference colonies suggesting that there were no adverse effects on chick growth and development or possible endocrine disruption in chicks from the AOC. Immune function assessed using the PHA skin response test was suppressed in herring gull chicks from a colony in New York with high levels of PCBs, 2,3,7,8-TCDD, and total TEQs in liver (Grasman *et al.* 2013). In this study, spatial patterns for immune function differed in the two study years between chicks from the AOC colony and the two reference colonies. In 2015, PHA response was significantly lower in chicks from the AOC colony compared to the Double Island reference colony but not the Lake Ontario reference colony. In 2016 when PHA response was lower in chicks from Double Island relative to 2015, no significant difference in estimates of the PHA stimulation index was found among the three study colonies. Consistent between years however is that immune function was not significantly different between chicks from the AOC colony and the Lake Ontario reference colony.

In conclusion, the results of this three-year study suggest that there is no evidence of impaired reproduction or deformities in herring gulls attributable to local contamination effects within the Hamilton Harbour AOC. Embryonic viability in artificially incubated herring gull eggs was consistently high (100%) and productivity at the AOC colony exceeded levels required to maintain a stable herring gull population. No embryonic deformities were evident in gull eggs incubated in the laboratory and no morphological deformities were found in 21-day-old herring gull chicks from the AOC colony. In addition, a low prevalence of morphological deformities (0.04%) was found in cormorant chicks at two AOC colonies in the one survey year and frequencies were not significantly different from the Lake Erie reference colony. Significantly higher concentrations of two persistent organochlorines, sum PCBs and *p,p'*-DDE, were found in gull embryos from the AOC colony compared to one reference colony in the two study years, a pattern likely due to several factors specific to the Harbour. Mercury concentrations in gull embryos were similar or lower than concentrations at reference colonies. Concentrations of organochlorines and mercury were largely similar between herring gulls (embryos) and cormorants (eggs), two colonial waterbird species with a close connection to the aquatic environment. Large declines in concentrations of sum PCBs and other organochlorines, mercury, 2,3,7,8-TCDD and total TEQs in herring gull eggs since the 1980s indicate that exposure to these compounds has decreased in herring gulls foraging in the AOC. Large decreases in organochlorine burdens were also found in double-crested cormorant eggs between 1989 and 2016. Overall, concentrations of PCBs and other organochlorine compounds, PBDEs, dioxins and furans, and mercury were not sufficiently elevated to adversely impact the reproductive success and development of herring gulls and cormorants nesting in the Hamilton Harbour AOC.

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