Reproductive Health and Development in Northern Leopard Frogs (*Rana pipiens*) in the St. Clair River Area of Concern (Ontario)

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Abstract

Reproductive health and development were examined in a four year study of northern leopard frogs (*Rana pipiens*) in the St. Clair River Area of Concern identified (in part) as a result of high concentrations of contaminants in the aquatic environment. Two exposure studies were conducted in the laboratory in which embryos were raised in water (2007) and sediment and water (2011) collected from several AOC locations to assess hatching success and frequencies of deformities in embryos. Hatching success of embryos was high (>98%) and frequencies of embryonic deformities were low to moderate (<7%) at AOC locations in both exposure studies with statistically similar frequencies reported at non-AOC Great Lakes reference sites. The prevalence of deformities in wild populations of newly-transformed froglets ranged widely based on 18 surveys at seven AOC locations over four study years (0-11.6%). Deformity frequencies at two AOC locations (Chematogan Channel and Bay Lodge) in 2007 were significantly higher compared to the reference sites where they also exceeded the 5% threshold which is considered elevated in wild amphibian populations. This finding was not repeated in subsequent surveys of Chematogan Channel and Bay Lodge in 2011 and/or 2014 where the prevalence of deformities was well below this threshold. Surveys of young-of-year frogs prior to hibernation revealed that the prevalence of male frogs with testicular ooctyes ranged widely in two study years (8.3-85.7%) where frequencies were significantly higher in 4 of 11 surveys at AOC locations compared to reference sites. Contaminant body burdens in these frogs were generally low for PCBs and other organochlorines and below concentrations associated with toxicity. Mercury body burdens were relatively higher in frogs and most notably at Bassett Channel and Goose Lake. Concentrations of PCBs, most other organochlorines, and mercury (estimated, as methylmercury) in frogs were below respective tissue residue guidelines for wildlife consumers. Total DDT was the one exception where exceedences of the guideline were noted in two pooled frog samples from Johnston Channel in 2006. Vitellogenin, a protein normally produced by females for egg production, was not detected in any male frogs from study sites. Concentrations of atrazine in water were low, varied among study sites, and were not directly associated with the increased prevalence of testicular ooctyes in males from AOC locations. Other agricultural herbicides and nutrients in water were below federal guidelines for the protection of aquatic life while concentrations of some trace metals, such as chromium and cadmium, occasionally exceeded these guidelines but were likely below those considered toxic to amphibians. In summary, no effects on reproduction and development were found in embryos raised in the laboratory and body burdens of organochlorines in wild populations of frogs were well below those associated with adverse effects on reproduction and development. Mercury concentrations were likely below effect levels however little is known with respect to potential effects associated with mercury exposure in amphibians. Deformity frequencies in froglets were low overall based on multiple surveys of many AOC locations over four years and also relative to frequencies reported from surveys conducted at other Great Lakes AOC locations. Elevated frequencies of males with testicular ooctyes at some AOC locations in this study were within ranges reported outside of the AOC and, as such, cannot be attributed to stressors within the AOC only. Overall the results of this study provide no evidence of impairment of reproductive health and development in wild populations of northern leopard frogs that can be attributed to high level of contaminants in the St. Clair River AOC (Ontario).

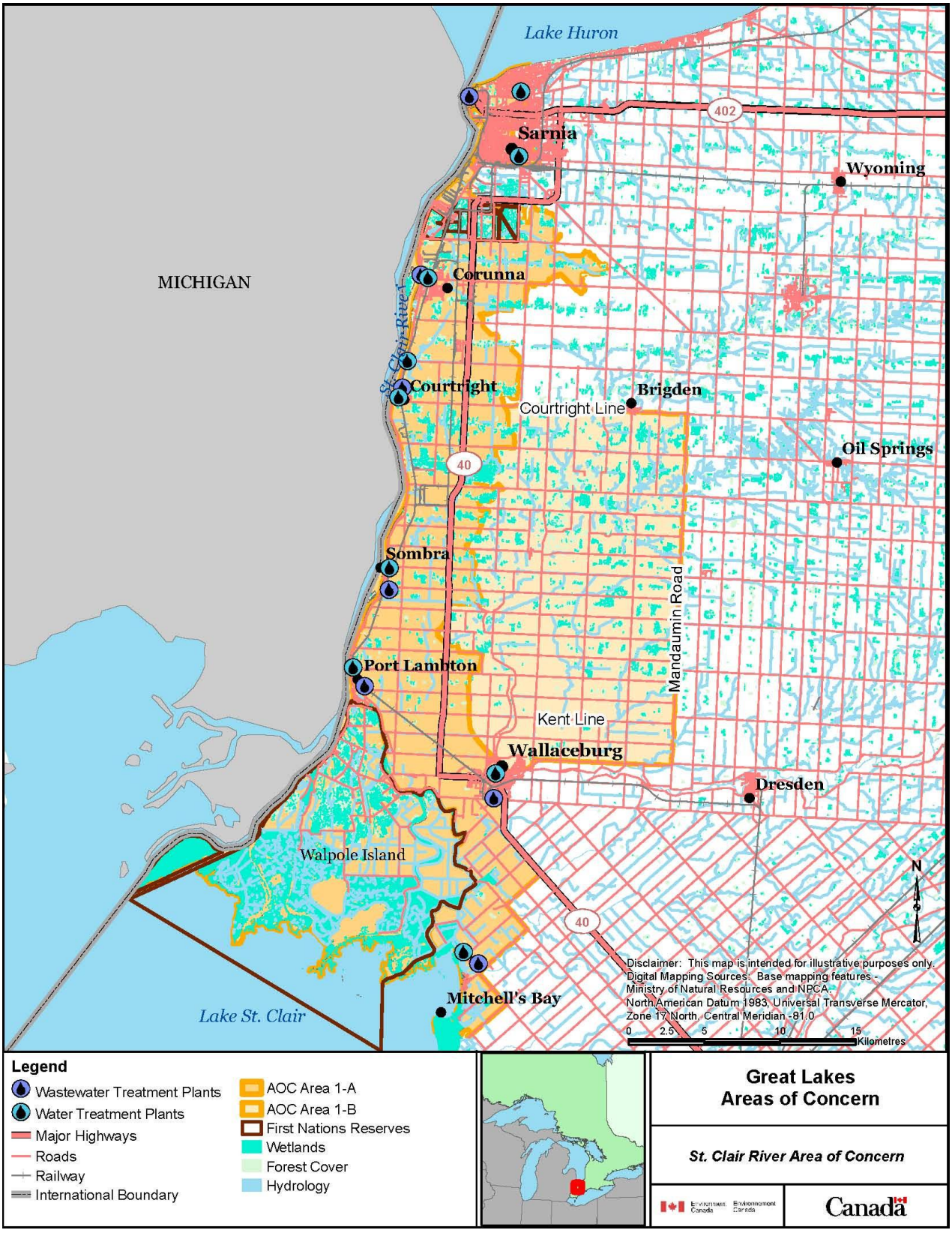
Introduction

As an important Great Lakes shipping channel and source of water for local industries, the St. Clair River is approximately 64 kilometres in length and flows in a southerly direction from the tip of Lake Huron and into Lake St. Clair. Prior to entering Lake St. Clair, the River branches into several channels creating an extensive delta known as the Walpole Delta which includes Walpole Island First Nations' Territory. The St. Clair River was designated by the International Joint Commission as one of 43 Great Lakes Areas of Concern (AOC) where environmental degradation was thought to be causing harm to the broader Great Lakes ecosystem. Fourteen beneficial use impairments (BUIs) relating to, for example, swimming, fishing, drinking water, fish and wildlife health and habitat, were used by the IJC as criteria to identify these problem areas in the Great Lakes. The binational AOC includes the main river, its delta channels, and coastal watersheds in Ontario and Michigan. Industrial and municipal activities and agricultural practices on both sides of the Canadian and American border contributed to localized degradation of the AOC as a result of point source discharges from municipal and industrial sources, non-point sources (e.g. urban storm water and rural runoff) and habitat loss (Ontario Ministry of Environment and Energy and Michigan Department of Natural Resources 1995). The eastern watershed draining from Ontario consists of several tributaries encompassing an area of approximately 20,976 hectares, of which 78% of the land area is agricultural (Ontario Ministry of Environment and Energy and Michigan Department of Natural Resources 1995; Figure 1).

Historical industrial, municipal and agricultural activities in the AOC have impacted water and sediment quality by introducing contaminants such as polychlorinated biphenyls (PCBs), mercury, pesticides, polycyclic aromatic hydrocarbons (PAHs), dioxins, oil and grease into the aquatic system (Ontario Ministry of Environment and Energy and Michigan Department of Natural Resources 1995). Many of these subsequently become available to biota, bioaccumulate in aquatic organisms and fish and then biomagnify through the food web. Exceedences of sport fish consumption guidelines for some compounds such as PCBs and mercury have resulted in restrictions on the consumption of several species of fish in different portions of the River (Ontario Ministry of Environment and Energy and Michigan Department of Natural Resources 1995). Elevated concentrations of PCBs and DDT were found in snapping turtle eggs (*Chelydra serpentina*) collected from Mitchell's Bay in the AOC in 1984 (Struger *et al.* 1993). In aquatic-feeding wildlife, increased contaminant exposure is frequently associated with reproduction and development problems (e.g., Bishop *et al.* 1998). As part of a large Great Lakes-wide study, this was examined in snapping turtle eggs collected from a nearby location on Lake St. Clair just outside of the AOC boundary in 2001-2004. While eggs from this location had significantly higher concentrations of numerous pesticides and PCBs relative to reference sites, no evidence of reduced hatching success or increased frequencies of hatchling deformities was found (de Solla *et al.* 2007, 2008). Further studies were recommended by Mayne (2008) to examine effects on reproduction and deformities associated with elevated contaminant exposure in wildlife collected from within the boundary of the AOC.

In order to address this issue, Environment Canada (EC), in collaboration with Walpole Island First Nation's Heritage Centre, initiated a four year study to assess the presence and degree of reproductive and developmental impairment in northern leopard frog (*Rana pipiens*) populations in the St. Clair River

Figure 1. The St. Clair River Area of Concern as defined by the boundary in Ontario.



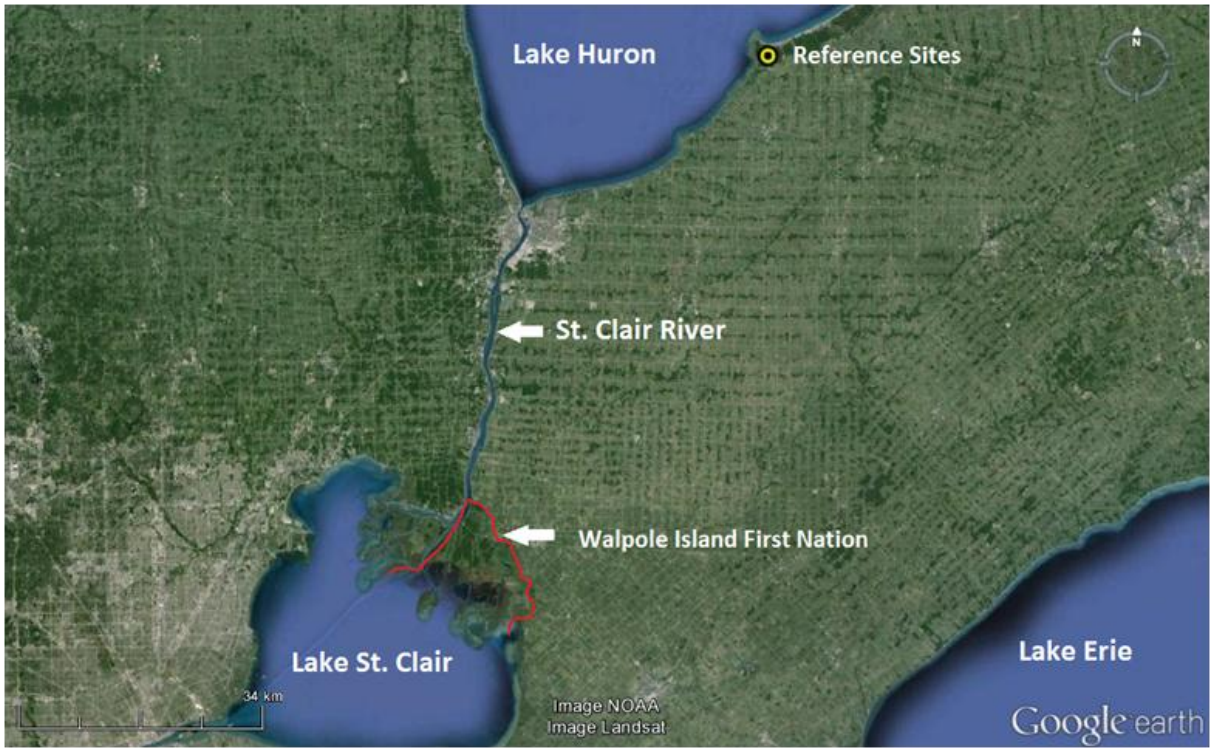
AOC. This species is ideal since it is widespread, native, semi-aquatic, and sensitive to poor water quality and the effects of contaminants. It has also been used in similar EC assessments of amphibian health in wetlands at other AOCs allowing for multi-site comparisons among AOCs. The potential for reproductive impairment in amphibians was examined in 2007 by exposing leopard frog eggs to water collected from multiple locations in the AOC to assess the ability of amphibian eggs to hatch and develop normally. In addition, studies have shown that amphibians can be exposed to contaminants in sediment which can cause changes in development or survivorship, particularly for embryos or larvae (Snodgrass *et al.* 2005; Peterson *et al.* 2009; Brand *et al.* 2010). Therefore since sediment can represent a significant source of contaminants to the aquatic environment, the exposure experiment was repeated in 2011 using water and sediment collected from known areas of contamination in the AOC to assess how sediment toxicity influences egg hatchability and the incidence of deformities in embryos. Capture and release surveys of newly-transformed froglets were conducted over four years (2006, 2007, 2011, and 2014) to determine if amphibian deformities were elevated at AOC locations compared to upstream reference sites. Reproductive impairment was also assessed by histological examination of reproductive organs and identification of structural abnormalities that might impair reproduction. Improper protein expression might be indicative of endocrine disruption and the presence of vitellogenin, a protein normally produced only in females, was also assayed for in blood of male frogs. Water was collected from study sites to assess potential exposure to agricultural pesticides, nutrients and trace metals in frogs. Contaminant body burdens were determined in frogs collected from several locations within the AOC and evaluated based on known harmful concentrations of contaminants.

Methods

Site Descriptions

Surveys and collections of northern leopard frogs, water collections for pesticide, nutrient and trace metals analyses as well as water and sediment collections for embryonic exposure studies were performed at a large number of locations within the St. Clair River AOC including locations in the Walpole Island First Nations' (WIFN) Territory in 2006, 2007, 2011 and 2014 (Figure 2). Study sites examined within WIFN included Bassett Channel, Goose Lake, Johnston Channel, Chematogan Channel, Dyke Road, Chenal Ecarte, Black Shack Road, St. Anne Island, and Heritage-White Bread. For the purpose of this study, the Heritage-White Bread site represents the same location on opposite sides of Chenal Ecarte with Heritage on the WIFN side surveyed in 2006 and White Bread across the channel (and not on WIFN Territory) surveyed in 2007. Other locations within the AOC but outside of WIFN included Bay Lodge at Mitchell's Bay and Bear Creek on Chenal Ecarte (part of St. Clair National Wildlife Area and within the AOC). In addition, water samples were collected from six AOC locations on the St. Clair River: Sarnia, Talfourd Creek, Baby Creek, Terra, Clay Creek, and Marshy Creek. Two reference sites were also selected on the south shore of Lake Huron located upstream of the St. Clair River AOC: Port Franks (at the mouth of Mud Creek and the Cut) and the Wood Road site which is a series of boating channels adjacent to Kettle Point First Nations (Figure 2). A complete listing of survey and sampling locations, coordinates and corresponding years of data collection for each parameter examined is provided in Appendix 1.

Figure 2. Study locations within the St. Clair River AOC and upstream Lake Huron reference sites (Port Franks and Wood Road) for northern leopard frogs in 2006, 2007, 2011, and 2014.

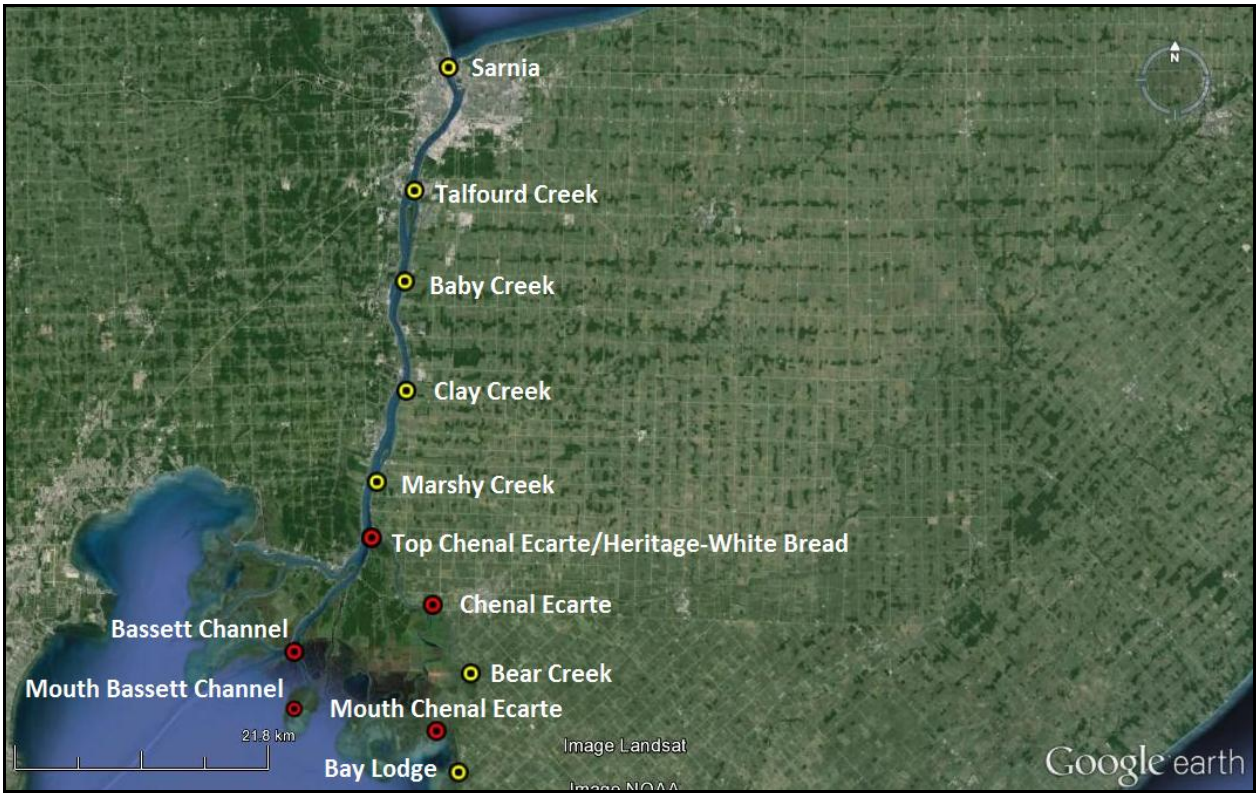


Embryonic Exposure Studies

The minimum requirement for amphibian reproduction in an area is that viable eggs deposited at a site can successfully develop into free swimming tadpoles. Fertilized northern leopard frog eggs were raised in water and sediment collected from locations within the St. Clair River AOC and upstream reference sites to assess the impact of AOC water quality and sediment quality on amphibian development. This is an adaptation of the standard FETAX test, which is used to assess water quality for both acute toxicity and teratogenicity; therefore, this test is ideal for the assessment of both reproductive impairment and deformities. Two separate embryonic exposure studies were conducted in the laboratory using northern leopard frog eggs exposed to: 1) water sampled from study sites in 2007 and 2) both water and sediment sampled from study sites in 2011. Eggs used in both studies were collected in the spring as fresh egg masses from a reference population, Beverley Swamp in Flamborough Township, Ontario (43°22'0.48" N, 80°6'58.88" W), previously used for deformity assessments and where eggs were readily available. This reference population was also chosen as a result of minimal influence from agricultural, industrial, or municipal runoff or effluent. Eggs were transported to the laboratory either at the St. Clair National Wildlife Area in 2007 or to Canada Centre for Inland Waters in Burlington in 2011 where exposures were started the following day. Noteworthy is that an in-situ exposure experiment was conducted initially in 2006 in which eggs were placed in cages in AOC wetlands; this study however was unsuccessful as a result of strong currents and rough waters.

For the water exposure study in 2007, approximately 30 eggs (ten from each of the three fresh egg masses) were raised in hexane-cleaned one liter glass jars containing 350 ml of fresh water collected from each of the study sites. Since access to sites on WIFN during the breeding season was limited in 2007, embryonic exposures were assessed using water from sites further upstream along the St. Clair River. These included eight St. Clair River AOC locations, Sarnia, Talfourd Creek, Baby Creek, Clay Creek, Marshy Creek, Heritage-White Bread, Bear Creek and Bay Lodge and the Lake Huron reference site, Wood Road (Figure 3). Five replicate jars containing 29-32 eggs per replicate were prepared for each site. Every other day, ten liters of water were collected using a sampling pole from each of the study sites, placed into chemically clean four liter glass jugs and transported back to the laboratory where they were stored at room temperature. The eggs were checked and the water changed every 24 hours in each of the replicates. The experiment was conducted at room temperature.

Figure 3. Study locations within the St. Clair River Area of Concern where samples were collected for the embryonic exposure studies of northern leopard frog eggs in 2007 and 2011. Yellow circles represent water collection locations in 2007. Red circles represent water and sediment collection locations in 2011. Note that the Heritage-White Bread water collection location (yellow circle) is hidden behind Top Chenal Ecarte (red circle).



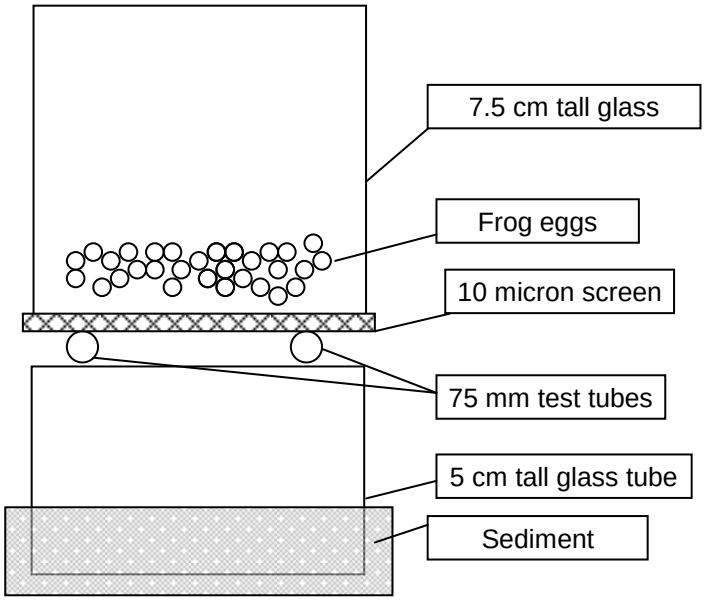
For the St. Clair River water and sediment exposure study, five St. Clair River locations were selected for sampling in 2011 based on chemical contamination in sediment which had been sampled previously in 2005 and reported in GLIER & DBS (2008). Sediment was sampled from three AOC locations on Chenal Ecarte (top, midstream, and mouth) and two locations on Bassett Channel (top and mouth; Figure 3).

Port Franks served as the reference site. Sediment samples were obtained using a mini-ponar and placed into clean glass jars. Forty liters of surface water samples were collected in bisphenol A-free 10 L carboys which had been cleaned by filling them with tap water and allowing them to sit for three days to allow any chemicals present in the plastic to leach out. Both sediment and water samples were refrigerated at 4°C until use (within one week).

Two hundred grams of sediment from each site were then weighed and placed into clean two liter wide-mouthed glass jars (I-Chem) to create five replicates per site. Two sets of these jars were made up to allow for replenishment of water every 24 hours which ensured that embryos continued to access well-oxygenated water and that they were not excessively exposed to their own waste products. It was also necessary that the water and sediment settle for 24 hours before eggs could be added to minimize suspension of solids.

Egg holders were constructed to prevent the eggs from coming into direct contact with the sediments and to protect them from becoming coated with sediment and dying due to lack of oxygen flow (Figure 4). Egg holders were constructed out of two glass tubes (Pegasus Glass), two 75 mm test tubes (Fisher Scientific), 10 micron screen, and 100% clear silicone rubber aquarium sealant (Marineland). One piece of glass tube was attached to two test tubes as spacers which were plugged up with aquarium sealant; this was allowed to cure for 24 hours. At the same time, another glass tube was attached to a round section of 10 micron screen using aquarium sealant and allowed to cure for 24 hours. These two pieces were then attached together using aquarium sealant with the screening and spacers in between.

Figure 4. Diagram of egg holder used to separate leopard frog eggs from sediments.



The day before eggs were obtained, one liter of water from the appropriate treatment was added to each replicate of one set of jars and allowed to settle for 24 hours before eggs in their holders were

added. The eggs in their holders were then introduced to the first set of jars. After this was completed, one liter of water from the appropriate treatment was added to a second set of jars and allowed to sit for 24 hours. On a 24 hour cycle, eggs in their holders were removed from one set of jars and placed into the other at which time 95% of the old water from the first set of jars was siphoned off using tubing specific to each treatment and replaced with fresh treatment water. This was allowed to sit for 24 hours when the same process was repeated. The experiment was carried out at room temperature.

In 2011, three leopard frog egg masses were collected from Beverley Swamp. Eggs were examined in a Petri dish of treatment water and separated and placed into replicate egg holders within one day of egg collection and within 24-48 hours of having been laid; dead eggs were not included in replicates. Between 9 and 12 eggs in total were cut from each egg mass and placed into each replicate following the Short Term Exposure of Frog Embryos in Vitro Standard Operating Procedure of the Canadian Wildlife Service for a total of 30-33 eggs per replicate.

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

Sediment sampled for use in this exposure study was chemically analyzed for organochlorines, polyaromatic hydrocarbons (PAHs), and trace metals by the Great Lakes Institute of Environmental Research (GLIER) at the University of Windsor in Windsor, Ontario to provide a more recent assessment of contaminant exposure in the AOC.

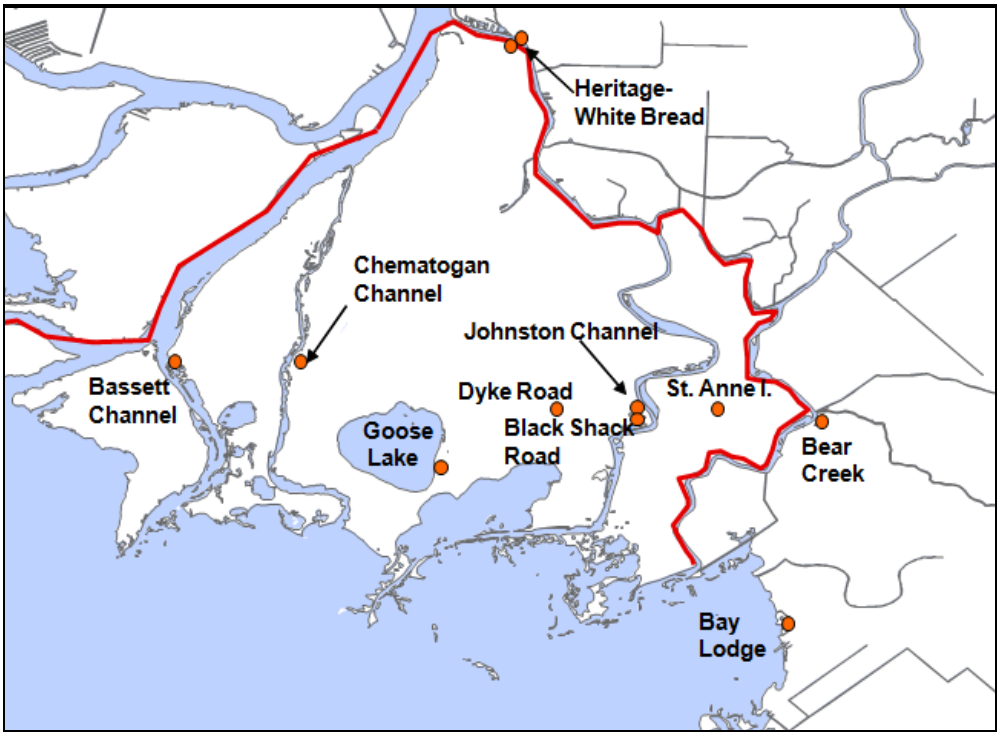
Deformities in Wild Populations of Newly-Transformed Froglets

Northern leopard frogs were selected as a model amphibian species for the assessment of gross skeletal deformities. They are an appropriate organism to assess deformity risks in aquatic ecosystems due to their semi-permeable skin, well-documented susceptibility to teratogenic chemicals, and a biphasic lifecycle which includes two stages of intense morphometric change. Optimally, deformity surveys occur when froglets have just completed metamorphosis and before they are exposed to additional predation and injury. Differential mortality from predation between deformed and non-deformed frogs could lead to an under-estimate of frequencies of deformities if surveys are performed too late in the season. In addition, while amphibians have the ability to regenerate from some injuries, regeneration is often incomplete resulting in the appearance of a deformity. This makes developmental deformities difficult to distinguish from those caused by injury. For this reason it is advisable to survey soon after the limbs have formed as they are the primary site of skeletal deformities.

Newly-transformed froglets were collected from AOC and reference study sites and assessed for deformities in early July of 2006, 2007, 2011 and 2014 (Figure 5). Frogs were collected by walking along the shoreline and captured using a dip net or by hand. Frogs were held in coolers of water at the site until they could be assessed. Frogs collected in 2006 and 2007 were weighed with a Pesola scale and measured (snout-vent length) with electronic Vernier calipers. Deformities in frogs were scored visually

as the presence of gross morphological deformities and did not include those associated with a recent traumatic injury. After sampling, frogs were released at the site of capture to reduce the impact on the breeding population. If sampling was to occur more than one day at a site, frogs were marked by branding with a length of copper wire cooled in dry ice to the white skin of the belly to avoid re-counting the same individuals after release. While an attempt was made to collect 100 frogs per site, this was not always possible with sample sizes ranging from 50-107 frogs per site in 2006, 42-135 frogs per site in 2007, 66-120 frogs per site in 2011 and 21-108 frogs per site in 2014.

Figure 5. Study locations within the St. Clair River AOC where northern leopard frogs were assessed for deformities, gonad histology and contaminant body burdens in 2006, 2007, 2011 and 2014. The red line denotes the boundary of the Walpole Island First Nations Territory.



For both egg collections and capture of frogs, scientific collection permits were obtained from the Ontario Ministry of Natural Resources and NWRC/CWS Animal Care permits were obtained from Environment Canada. Both sampling gear and sampling clothing were rinsed with a solution of chlorine and water to kill any potential pathogens following a survey before a new site was visited.

Gonad Histology and Vitellogenin-like Protein

Maturing pre-hibernation young-of-year northern leopard frogs were collected and assessed for the prevalence of gonadal deformities in the fall when gonads were fully sexually differentiated and had reached a stage of maturity which allowed for a more complete histological assessment. Frogs were captured by dip net at five and six St. Clair River AOC locations in September of 2006 and October of 2007, respectively, as well as the two reference sites. Upon capture, blood was sampled for the presence of vitellogenin-like protein in plasma. The presence of vitellogenin in male frogs is indicative of

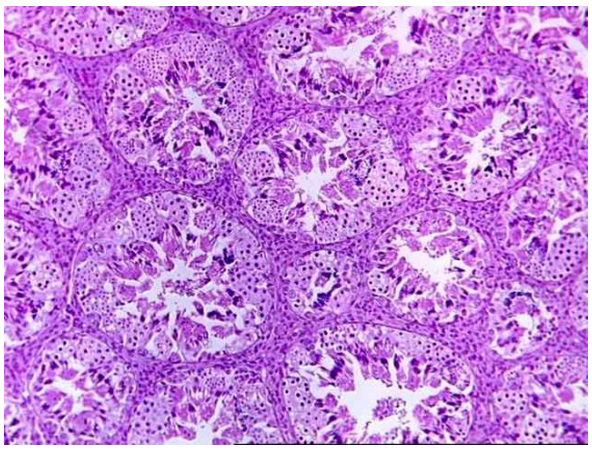
exposure to environmental estrogens since vitellogenin is an egg yolk protein produced only by females and not normally by males. Captured frogs were held in large plastic containers for a maximum of two hours prior to being blood sampled to reduce capture stress and to standardize this minimal stress between sites. Frogs were anaesthetized in a 0.01% solution of MS-222 and blood was collected via cardiac puncture using a heparinized syringe. Blood was collected and assayed for the presence of vitellogenin-like protein in male frogs from the following study sites (sample sizes shown in brackets for 2006, 2007): Bear Creek (10, 10), Goose Lake (10, 7), Bay Lodge (9, 7), Johnston Channel (11, 8), Chematogan Channel (0, 6) and Bassett Channel (1, 3) and two reference sites, Wood Road (13, 7) and Port Franks (2, 8). Details of methods used for the detection of the vitellogenin-like protein in blood are found in McDaniel *et al.* (2008).

Following collection, frogs were transported to the laboratory where they were euthanized in a 1.0% solution of buffered MS-222. Frogs were sexed by examination of their gonads under a dissecting microscope. The kidney/gonad complex was removed and preserved for histological assessment. The remainder of the frog was then submitted for analysis of contaminant body burdens (see below).

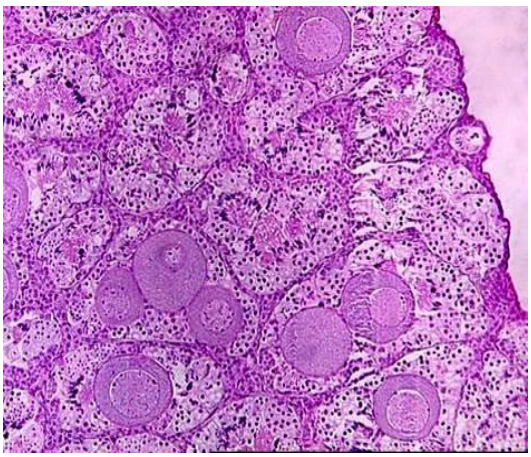
Gonads of all males, a sub-sample of females and those whose sex was ambiguous were sent to the Animal Health Laboratory at the University of Guelph in Guelph, Ontario for histological processing. Testes were fixed in Bouin's solution (Sigma-Aldrich) for 24 hours to increase histological resolution then transferred to 10% buffered formalin (Fisher Scientific). Testes were dehydrated in an alcohol series and embedded in paraffin prior to sectioning. Testes were oriented laterally and six to eight serial sections of 7.0 µm in thickness were taken at two depths; near the top of the gonad and at a depth of approximately 350 µm. At least two sets of serial sections were taken at the two depths and a third set taken if the presence of oocytes was unclear. Slides were stained with Harris' Haematoxylin and Eosin and all testes sections were examined under a Zeiss compound microscope (Carl Zeiss Canada, Ltd) and scored for the presence of gonadal abnormalities. Specifically in males, gonads were examined for the presence of testicular oocytes which are eggs found embedded within otherwise normal testicular tissue (Figure 6).

Figure 6. Histology of a normal testes tissue in a male leopard frog (a) showing many seminiferous tubules where sperm develop. Histology of abnormal tissue containing testicular ooctyes (b) where some seminiferous tubules contain eggs and where the testes are also producing sperm.

a) Normal testes tissue containing sperm



b) Abnormal testes containing testicular oocytes



Chemical analyses of whole bodies of frogs (minus gonads and spleens) were conducted at the National Wildlife Research Centre (NWRC) in Hull, Québec and at GLIER. In 2006, organochlorines contaminants and mercury were measured at NWRC in frogs collected from four AOC locations, Bassett Channel, Goose Lake, Johnston Channel, Heritage-White Bread and the two upstream reference sites on Lake Huron, Wood Road and Port Franks. In 2007, frogs were collected from three additional AOC locations, Chematogan Channel, Bear Creek and Bay Lodge, and analyzed for organochlorines at GLIER and for mercury at NWRC. For organochlorine analyses, frogs were analyzed as pools consisting of two same sex frogs with 5-7 pools of frogs analyzed per site in the two study years. Chemical analyses for mercury were performed using individual frogs (i.e., not as pools) in 2006 and 2007 with nine or ten individuals analyzed per site.

Organochlorine contaminants measured included *p,p'*-DDE (dichlorodiphenyldichloroethylene), *p,p'*-DDT (dichlorodiphenyltrichloroethane), *p,p'*-DDD (dichlorodiphenyldichloroethane), oxychlordane, *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, hexachlorobenzene (HCB), dieldrin, heptachlor epoxide (HE), octachlorostyrene (OCS), mirex, and polychlorinated biphenyls (PCBs). Samples at NWRC were analyzed according to the methods of Norstrom *et al.* (1988). Frog samples were thawed and homogenized and a 5 g aliquot was taken for analysis. Samples underwent neutral extraction, removal of lipids and biogenic compounds by gel permeation chromatography, followed by further cleanup using florisil column chromatography. Quantitative analysis of organochlorine compounds was performed using capillary gas chromatography using a mass selective detector (GC/MSD) operated in selected ion monitoring mode. The first injection was designed to determine organochlorines using an external standard method on the basis of ion response factors in a standard solution containing 21 organochlorines. The second injection was performed to determine PCBs using an external standard method with a standard mixture of Arochlor 1242:1254:1260 (1:1:1). Brominated flame retardants (BFRs) including polybrominated diphenyl ethers (PBDEs) were quantified in the third injection of the GC/MSD chemical analysis in the NICI mode. Sum polybrominated diphenyl ethers reported are based on the sum of concentrations of 14 congeners found above the limit of detection (IUPAC# 17, 28, 47, 49, 66, 85, 99, 100, 138, 153, 154/BB-153, 183, 190, and 209). Concentrations of two other brominated flame retardants, BB-101 and hexabromocyclododecane (HBCDD), are also presented where found above the detection limit. The percent recovery of $^{13}C_{12}$-labelled internal standard of tetra-, penta-, hexachlorobenzene and DDE ranged from 51.4% to 96.8% with a mean percent recovery of 83.6%. The percent recovery of $^{13}C_{12}$-labelled internal standard of six PCB congeners ranged from 94.0% to 104.2% with a mean percent recovery of 99.1%. For samples analyzed at GLIER in 2007, organochlorine contaminants were also quantified using GC/MSD using one standard mixture of Arochlor 1242:1254: 1260 (1:1:1) for quantifying PCBs and two standard solutions for organochlorines. BFRs were not measured in 2007 samples. The percent recovery of tribromobenzene used to measure sample recovery efficiency at GLIER ranged from 63.0% to 95.1% with a mean recovery of 82.7%. Double-crested cormorant (*Phalacrocorax auritus*) egg reference material was used in both laboratories and analyzed with each run for quality assurance purposes. Samples were not adjusted for recoveries. Sum PCB concentrations were based the sum of 62 individual PCB congeners at NWRC in 2006 samples and 34 PCB congeners at GLIER in 2007. A comparison of sum PCB concentrations in 2006 samples using the 30 PCB congeners common to both laboratories revealed that sum PCBs calculated were 100% equal in

concentration in 29 of 30 samples analyzed thereby demonstrating that the contribution of these 32 additional congeners (which were generally non-detectable) had little influence on the total sum PCBs calculated; thus, sum PCB concentrations are largely comparable using the results from the two laboratories. Detection limits (DL) also differed between the two laboratories and were equal to 0.1 ng/g for organochlorines and BFRs at NWRC and 0.03 ng/g at GLIER in 2007. As a result where concentrations in 2007 samples were found below the 2006 DL of 0.1 ng/g, values were adjusted to one-half of the detection limit (i.e., 0.05 ng/g) in order to facilitate comparisons of concentrations among sites between the two years. Organochlorine concentrations are reported on a wet weight basis in ng/g.

Chemical analysis for mercury was conducted at NWRC where frog samples were freeze-dried, homogenized and weighed out directly into nickel combustion boats. Total mercury was analyzed using an Advanced Mercury Analyzer (AMA-254) equipped with an ASS-254 autosampler for solid samples according to CWS Method No. MET-CHEM-AA-03F. Analytical accuracy was monitored through the use of Standard Reference Materials in both study years which included DOLT-3, and TORT-2 (lobster hepatopancreas) from the National Research Council and Oyster Tissue 1566b from the National Institute of Standards and Technology. Percent recoveries of reference materials ranged from 96.4% to 110.0% with a mean percent recovery of 101.9% for mercury (N=27 samples). Recoveries of reference materials were within the certified range for all methodologies. Analytical precision for mercury analyses determined as percent Relative Standard Deviation (% RSD) of replicate samples of the same aliquot was generally below 6%. The detection limit of mercury was equal to 0.006 µg/g (dry weight). Mercury concentrations were determined on a dry weight basis and then converted to wet weight based on percent moisture content.

Water Chemistry – Pesticides, Nutrients and Metals

As a measure of water quality and to assess potential exposure in frogs, pesticides/herbicides, nutrients and trace metals were measured in water collected in 2006 and 2007 from 14 AOC locations (including WIFN sites) and the two upstream Lake Huron reference sites. Generally, three different time periods of water collection for herbicide and nutrient analysis were performed to coincide with measurements of biological endpoints in the two study years (i.e., in May when eggs were assessed, in June/July when deformities were assessed, and in September/October when gonads were assessed). While an attempt was made to collect water once during each of the time periods, this was not always possible and as a result, the number of sites sampled and the frequency of sampling varied between years. Water collections, for instance, were conducted in May 2007 only at Sarnia, Talfourd Creek, Baby Creek, Clay Creek, and Marshy Creek on the St. Clair River. In addition, a problem at the laboratory resulted in the loss of nitrate and ammonia data for water collections in May 2006. Trace metal analyses were conducted on water samples collected during one sampling period each study year, i.e., June 2006 and May 2007, at AOC and reference study sites.

Water was collected using a hand held sampling device approximately 18 cm below the surface. For pesticide residue analysis, water was immediately preserved at pH<2.0 for the fixation of acid herbicides (2-4 mL of a 50% solution of H_2SO_4 per 100 mL sample). For the analysis of total phosphorus, water samples were immediately preserved with 1 mL of 30% H_2SO_4 per 100 mL sample. Samples were not

filtered prior to analysis. Water samples were held at 4°C and transported to the Environment Canada's National Laboratory for Environmental Testing in Burlington, Ontario, where they were analyzed for nutrients, pesticides and trace metals using gas chromatography/mass spectrometry (GC/MS) or inductively coupled plasma (ICP) mass spectrometry. For a detailed description of analytical methods see Environment Canada (1997a,b,c,d). Pesticides were extracted with dichloromethane (100 mL per liter of sample). Nutrients quantified included nitrates/nitrites, ammonia and phosphate. Individual phenoxy-acid herbicides quantified included clopyralid, dicamba, 2,4-D (2,4-dichlorophenoxyacetic acid), 2,4-DB (4-(2,4-dichlorophenoxy) butyric acid), 2,4-DP (2-(4,4-dichlorophenoxy)-propionic acid), MCPA (4-chloro-2-methyl phenoxy acetic acid), MCPB (4-(4-chloro-2methyl-phenoxy)butyric acid), 2,3,6-TBA (2,3,6-trichlorobenzoic acid), 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), bromoxynil, Silvex, Picloram, Mecoprop, Imazamethabenz-methyl(A) and (B), and Imazethapyr (IPYR). Neutral herbicides quantified included butylate, desethylsimazine, desethylatrazine (D-atrazine), trifluralin, diallate-1, diallate-2, simazine, atrazine, triallate, metribuzin, metolachlor, dichlofopmethyl (hoegrass), and benzoylprop-ethyl (Endaven). Organophosphate pesticides quantified in 2006 included: naled, phorate, dimethoate, terbufos, fonofos, diazinon, disulfoton (Disyston), malathion, chlorpyrifos (Dursban), parathion, ethion, phosmet (Midan), and azinphos-methyl. Since concentrations of all organophosphates were found below the level of detection at all sites in 2006, these analyses were not repeated in 2007. For context, concentrations of nutrients, trace metals and pesticides were compared to benchmark values based on the Canadian Water Quality Guidelines (CWQGs) for the protection of aquatic life (CCME 2007).

Statistical Analyses

Analysis of Variance (ANOVA) tests were performed to examine spatial differences in mean contaminant concentrations and biological endpoints followed by Tukey's HSD post-hoc tests where ANOVA tests were significant. If data failed the assumption of equal variance (Levene's Test), comparisons were made using a Kruskal Wallis one way analysis of variance by ranks followed by non-parametric multiple contrast tests for unequal sample size (Zar 1984). Concentrations of compounds found below the limit of detection were given a concentration of one-half of the detection limit for calculations of mean and median values. Due to the overall low number of deformed froglets found at study sites, the Fisher exact test (one-tailed) was used to compare counts of deformed and non-deformed individuals at each AOC location with those at the two reference sites (which were pooled if appropriate). The Fisher exact test was also used to examine the prevalence of testicular oocytes in males from AOC locations versus pooled reference sites.

Results

Embryonic Exposure Studies

Hatching success was extremely high for embryos raised in water from eight St. Clair River AOC locations and the Wood Road reference site in 2007 with mean hatching success of eggs ranging from 98.0% at Sarnia to 100% at Heritage-White Bread and Bear Creek (Figure 7a). Overall, no significant difference in hatching success was found among sites ($F_{8,36}$=0.49, p=0.85). In addition, mean frequencies of embryonic deformities were low ranging from 0% at Marshy Creek and Bay Lodge to 2.1% at Talfourd Creek and also did not differ significantly among sites ($F_{8,36}$=0.76, p=0.64; Figure 7b).

Figure 7a. Mean hatching success (SD) of northern leopard frogs in 2007 raised in water from eight St. Clair River AOC locations and one upstream reference site on Lake Huron (Wood Road). Five replicates of 29-32 eggs were tested per site.

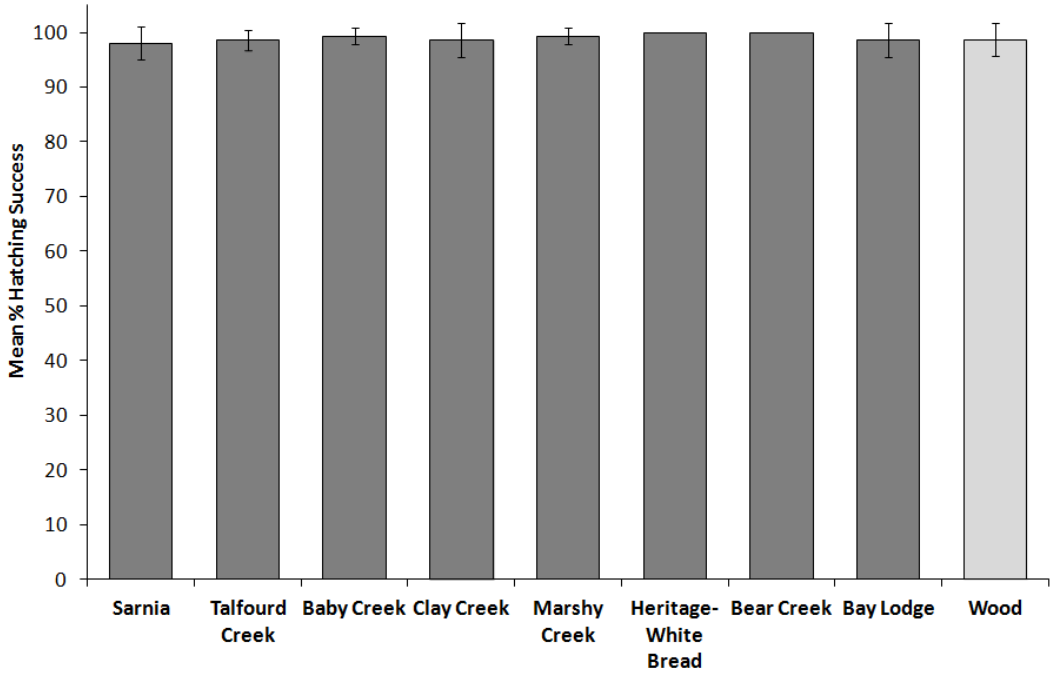
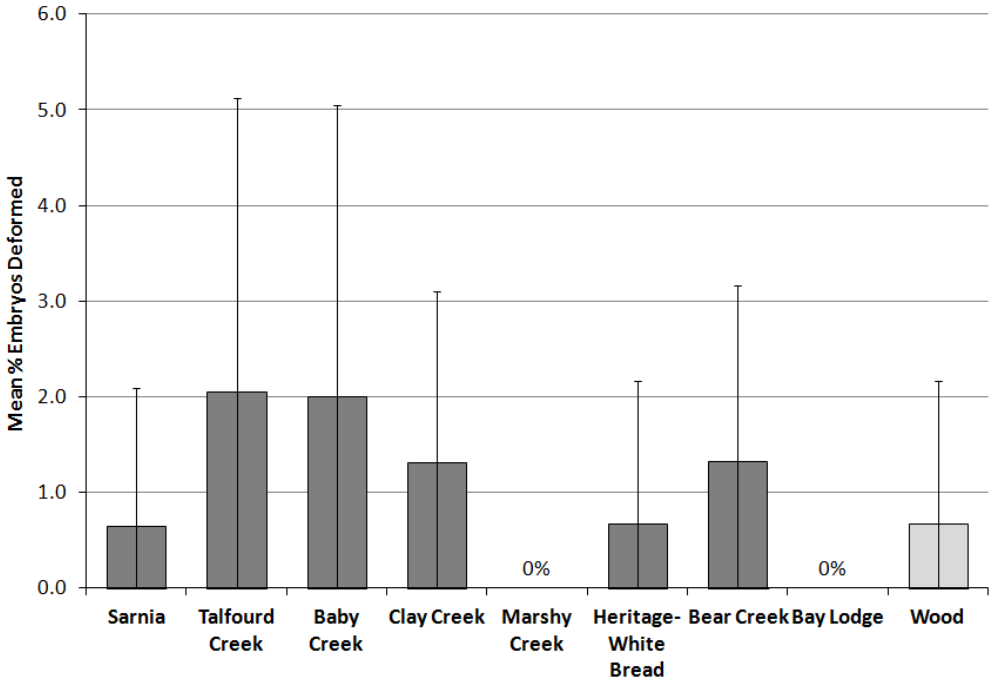


Figure 7b. Mean frequencies of deformities (SD) in northern leopard frog embryos in 2007 raised in water from eight St. Clair River AOC locations and one upstream reference site on Lake Huron (Wood Road).



Mean hatching success was also high in embryos exposed to sediment and water collected from five St. Clair River AOC locations and the Port Franks reference site in 2011 (range=98.8% to 100%; Figure 8a). No significant difference in hatching success was found among sites ($F_{5,24}$=1.39, p=0.26). Frequencies of deformities in embryos were low to moderate ranging from 3.8% at the Port Franks reference site to 7.0% at the mouth of Bassett Channel (Figure 8b). No significant difference in the incidence of embryonic deformities was found among study sites ($F_{5,24}$=1.04, p=0.42). The most common deformities were lateral flexures of the tail accounting for 20.6% of deformities, followed by stunted growth (15.6% of deformities), incompletely developed eyes (12.1%), asymmetries and dorsal flexures of the tail (10.6% each), gut formation or improperly coiled guts (9.9%), head and abdominal edemas (6.4% each), blisters (2.8%), and missing eyes and skin deformities (2.1% each). Appendix 2 provides a detailed summary of the types of deformities observed for each deformed embryo following exposure to water and sediment from study sites.

The results of chemical analyses of sediment collected in 2011 and used in the water and sediment frog exposure study are presented in Table 1. Sediment from the mouth of Chenal Ecarte had the highest concentrations of sum PCBs (34.88 ng/g) and *p,p*'-DDE (2.54 ng/g) which exceeded respective federal Interim Sediment Quality Guidelines (ISQGs) associated with the protection of aquatic life (CCME 2002). HCB and OCS concentrations were largely comparable among AOC locations. Sediment from the mouth of Bassett Channel most frequently exceeded ISQGs for polycyclic aromatic hydrocarbons (PAHs) with concentrations which were frequently nearly two times higher than concentrations at other study sites. Concentrations of acenaththylene (AL), phenanthrene (PHE), pyrene (PY), chrysene and triphenlyene (C&T) and Dibenz(a,h)anthracene (D(ah)A) exceeded respective ISQGs at most AOC locations. Mercury and arsenic were the only two of 19 metals measured in which concentrations exceeded respective guidelines at study sites. The highest mercury concentrations were found in sediment from the mouth of Bassett Channel (0.66 µg/g) and the mouth of Chenal Ecarte (0.63 µg/g) where concentrations also exceeded the 0.486 µg/g federal Probable Effect Level (PEL) associated with the protection of aquatic life (CCME 2002); the remaining three AOC locations exceeded the lower ISQG of 0.17 µg/g. The fewest sediment quality guideline exceedences were found in sediment from the top of Chenal Ecarte and the Port Franks reference site.

In order to examine effects in embryos associated with sediment toxicity, a Sediment Quality Index (SQI) score was calculated for each study site. This index combines individual contaminant data and provides an integrated numerical score for each site based on the number of sediment quality guideline exceedences and the magnitude of these exceedences. In total, concentrations of organochlorines, PAHs, mercury and trace metals for which there are federal guidelines were used to calculate SQI scores (22 compounds in total; Table 1c). SQI scores using both the ISQGs and more stringent PELs are shown. Based on ISQGs, the highest SQI scores (i.e., highest sediment quality) were found in sediment from Port Franks and the top of Chenal Ecarte while the lowest SQI score was found in sediment from the mouth of Bassett Channel where the highest mercury concentration and greatest number of ISQG exceedences were found. Based on PELs, few guideline exceedences were found and, accordingly, relatively higher SQI scores were calculated. No significant correlations were found between mean hatching success and either of the SQIs or mean frequencies of embryonic deformities and SQI-PELs calculated for each site.

Figure 8a. Mean hatching success (SD) of northern leopard frogs in 2011 raised in water and sediment collected from five St. Clair River AOC locations and one upstream reference site on Lake Huron (Port Franks). Five replicates of 30-33 eggs were tested per site.

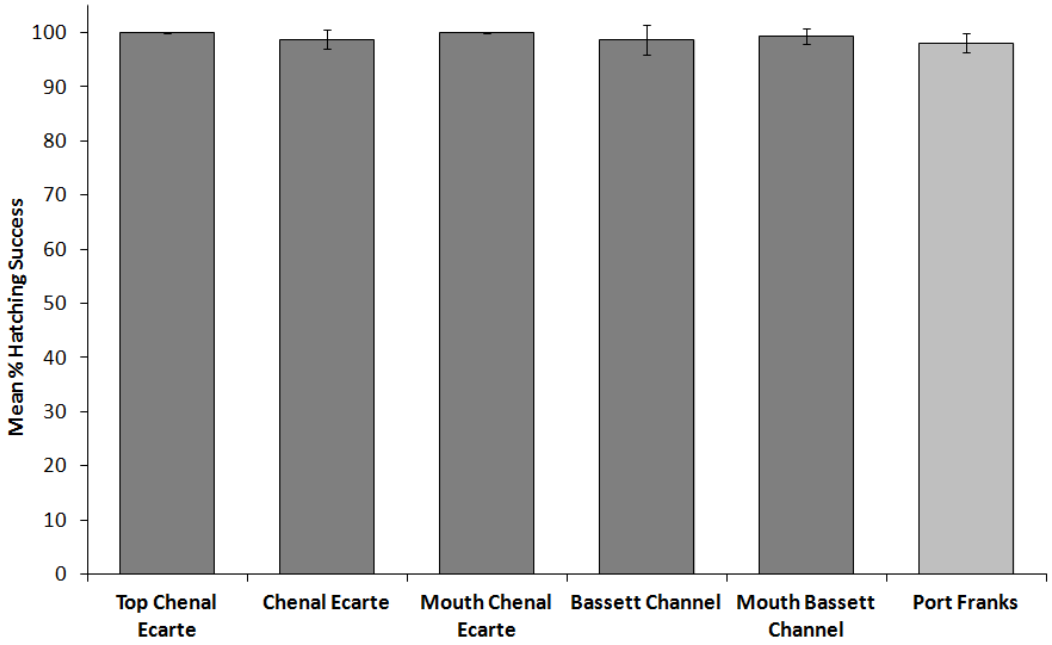


Figure 8b. Mean frequencies of deformities (SD) in northern leopard embryos in 2011 raised in water and sediment collected from five St. Clair River AOC locations and one upstream reference site on Lake Huron (Port Franks).

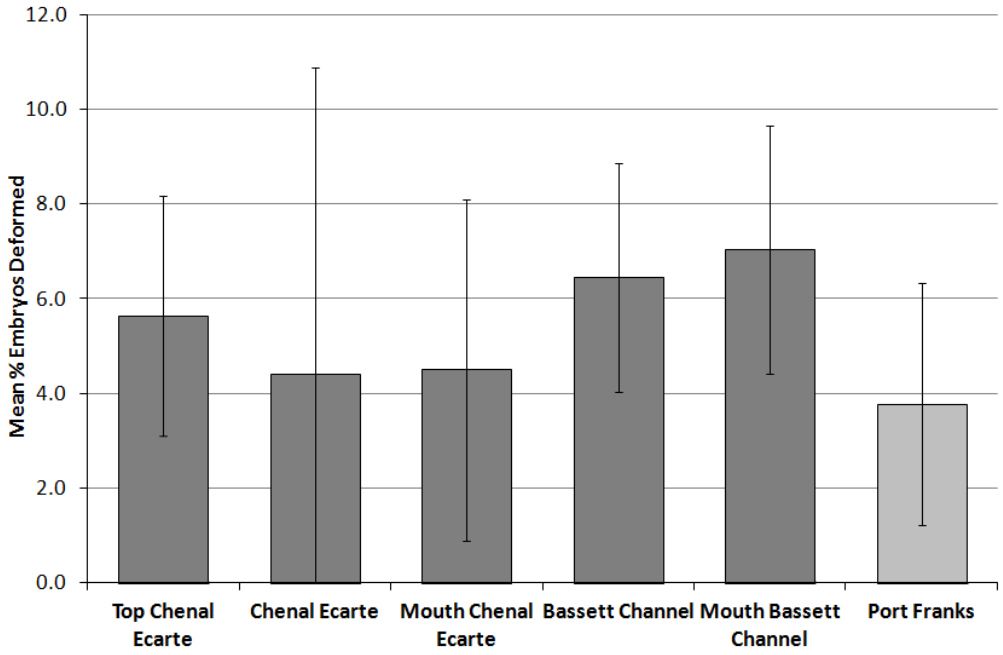


Table 1. Concentrations of organochlorines (a), polycyclic aromatic hydrocarbons (b), and mercury and trace metals (c) in sediment from five St. Clair River AOC locations and one upstream reference site (Port Franks) and associated Canadian sediment quality guidelines for the protection of aquatic life (i.e., Interim Sediment Quality Guideline (ISQG) and Probable Effects Level (PEL); CCME 2002). For compounds marked with a "†", no federal sediment guidelines are available and Ontario sediment quality guidelines have been used (i.e., Lowest Effect Level (LEL) and Severe Effect Level (SEL); Persaud *et al.* 1993). Sediment Quality Index (SQI) scores were calculated using organochlorine, polycyclic aromatic hydrocarbons, mercury and trace metal data shown here and for which federal guidelines are available (22 compounds in total).

a) Organochlorines (ng/g dry weight)

Site	Sum PCBs	HCB†	OCS	Sum Chlordane	*p,p'*-DDE
Top Chenal Ecarte	17.60	7.00	3.81	0.14	0.02
Chenal Ecarte	20.40	6.03	3.46	9.26**	1.63*
Mouth Chenal Ecarte	34.88*	2.80	2.88	1.21	2.54*
Bassett Channel	15.11	4.96	3.76	3.32	0.02
Mouth Bassett Channel	14.24	3.93	4.39	0.09	0.02
Port Franks	0.19	0.02	0.02	1.26	0.02
ISQG	34.1	20		4.50	1.42
PEL	277	24000		8.87	6.75

b) Polycyclic aromatic hydrocarbons (ng/g dry weight)

Site	NA	AL	AE	FL	PHE	AN	FLT	PY	B(a)A	C&T
Top Chenal Ecarte	6.38	7.57*	6.80*	18.71	81.87*	15.30	41.75	56.95*	16.11	35.80
Chenal Ecarte	17.74	8.49*	5.89	16.53	77.12*	13.90	82.51	85.28*	34.92*	65.29*
Mouth Chenal Ecarte	10.78	6.93*	5.16	14.83	52.26*	10.61	57.12	89.14*	29.01	60.07*
Bassett Channel	15.74	7.63*	5.45	16.88	77.96*	18.51	75.92	119.57*	50.04*	93.67*
Mouth Bassett Channel	31.69	14.58*	11.72*	30.10*	127.87*	34.43	134.22*	210.83*	85.51*	153.67*
Port Franks	0.61	1.01	0.37	1.54	11.86	2.60	22.69	18.59	8.62	11.24
ISQG	34.6	5.87	6.71	21.2	41.9	46.9	111	53	31.7	57.1
PEL	391	128	88.9	144	515	245	2355	875	385	862

Site	B(b)F	B(k)F	B(a)P	IP	D(ah)A	B(ghi)P	Total PAHs
Top Chenal Ecarte	11.16	4.75	10.03	7.66	1.99	14.99	337.80
Chenal Ecarte	34.98	16.49	30.94	27.01	8.39*	43.94	569.42
Mouth Chenal Ecarte	27.85	9.44	21.27	19.01	7.01*	29.71	450.18
Bassett Channel	42.93	16.81	47.60*	29.58	14.57*	50.92	683.77
Mouth Bassett Channel	68.60	28.19	83.28*	51.59	27.54*	90.11	1183.95
Port Franks	7.06	4.36	8.39	5.62	1.22	6.92	112.72
ISQG			31.9		6.22		
PEL			782		135		

c) Mercury and trace metals (µg/g dry weight)

Site	Hg	Al	As	Bi	Ca	Cd	Co	Cr	Cu	Fe
Top Chenal Ecarte	0.45*	2159.26	1.60	1.38	17603.53	0.24	2.12	12.65	4.11	3646.01
Chenal Ecarte	0.27*	3205.95	1.69	1.01	17080.54	0.24	2.70	15.61	8.02	4419.74
Mouth Chenal Ecarte	0.63**	5478.03	1.45	1.52	16231.79	0.23	3.36	21.74	10.89	5521.79
Bassett Channel	0.40*	2732.00	10.37*	17.45	18959.30	0.27	2.61	15.13	7.79	4217.11
Mouth Bassett Channel	0.66**	2356.09	9.28*	15.47	15761.27	0.25	2.51	13.87	9.55	3889.63
Port Franks	0.15	1347.14	6.25*	11.68	17069.10	0.13	0.97	8.07	2.53	1936.14
ISQG	0.17		5.9			0.6		37.3	35.7	
PEL	0.486		17			3.5		90	197	

Station ID	K	Mg	Mn†	Na	Ni†	P	Pb	V	Zn	SQI-ISQG	SQI-PEL
Top Chenal Ecarte	436.12	9088.43	99.31	78.53	4.68	114.81	4.06	10.81	19.03	82	100
Chenal Ecarte	572.15	8679.52	101.33	79.33	6.85	176.90	4.43	11.00	23.07	69	97
Mouth Chenal Ecarte	906.74	7529.77	109.36	81.57	10.16	217.84	6.57	14.64	29.57	71	97
Bassett Channel	527.00	14.21	98.46	84.85	6.16	144.78	4.89	10.49	26.93	66	100
Mouth Bassett Channel	398.34	11372.66	85.67	73.22	5.80	133.11	5.99	9.36	22.06	49	97
Port Franks	179.49	8823.79	44.64	77.81	2.00	62.52	0.99	6.28	4.31	97	100
ISQG			460		16		35		123		
PEL			1100		75		91.3		315		

*Sediment concentration exceeds Canadian ISQG or Provincial LEL

** Sediment concentration exceeds Canadian PEL or Provincial SEL

However, a marginally significant negative correlation was found between the mean frequency of embryonic deformities and the SQI-ISQG at the six study sites (Spearman rank correlation, r_s=-0.77, p=0.07). Upon examining mercury alone, a marginally significant positive correlation was found between the mean frequency of embryonic deformities and mercury concentration in sediment at the six study sites (Spearman rank correlation, r_s=0.77, p=0.07).

Deformities in Wild Populations of Newly-Transformed Froglets

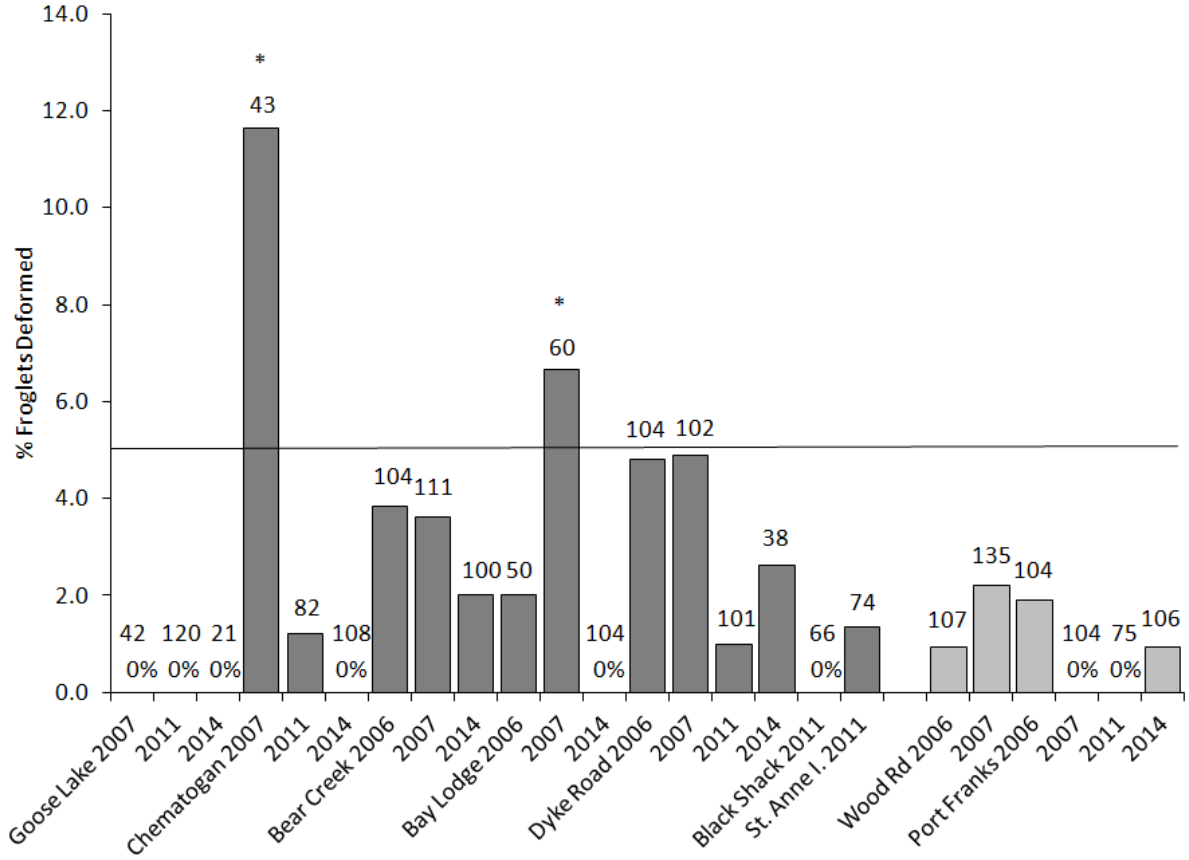
In 2006, the prevalence of deformities in newly-transformed froglets ranged from 0.9% at the Wood Road reference site to 4.8% at Dyke Road (Figure 9). Since the prevalence of deformities in froglets from the two reference sites was not significantly different, these were pooled for subsequent analysis (Fisher exact test, two-tailed, p>0.05). While there appeared to be a trend toward relatively higher frequencies of deformities in frogs from AOC locations, individual site-comparisons with the pooled reference sites were not significant (Fisher exact test, p>0.05 for all sites).

In 2007, the prevalence of deformities in froglets from AOC locations ranged from 0% at Goose Lake and Port Franks to 11.6% at Chematogan Channel (Figure 9). Deformity frequencies at the two reference sites were not significantly different and were pooled for subsequent analysis (p>0.05). Overall, the incidence of deformities was significantly higher at two of the six AOC locations, Chematogan Channel (Fisher exact test, p=0.0026) and Bay Lodge (Fisher exact test, p=0.032), compared to the pooled reference sites. In 2006 and 2007, the most common deformities were skeletal and affected the hind or forelimbs, usually involving missing digits or parts of digits. In more severe cases, particularly at Chematogan Channel, limbs were either absent or greatly malformed involving the fusing or reduction of several bones. Other deformities included missing or malformed eyes.

In 2011, deformity frequencies in froglets from AOC locations were low ranging from 0% at Goose Lake, Black Shack Road, and Port Franks to 1.4% at St. Anne Island (Figure 9). No significant differences in the prevalence of deformities were found between AOC locations and the Port Franks reference site. Deformities in frogs included abdominal edema, a missing eye and one frog with a large lesion where its tail would have been located. A notable finding in 2011 was the high prevalence of frogs from AOC locations with red spots and swelling of the feet and/or legs which were found primarily in the hind limbs. Specifically this was found in froglets from Goose Lake (13 of 120 frogs or 10.8%), St. Anne Island (4 of 74 frogs or 5.4%) and Dyke Road (2 of 101 frogs or 2.0%). Of the 19 St. Clair River AOC froglets showing these symptoms, one frog from Goose Lake also had a deformity (stunted rear right foot). As a conservative measure, this frog was not included as a deformed individual in the calculation of deformity frequency for Goose Lake frogs.

Symptoms of red spots and swollen feet/legs evident in froglets from three of five AOC locations in 2011 were similar to those found in froglets from Riverside Park in the Detroit River AOC in the same year. At that site, 32 out of 132 individuals (24.2%) also had red and swollen feet and/or legs, symptoms of which are indicative of an infection. Twelve of the Detroit River frogs were subsequently collected and sent to the Canadian Cooperative Wildlife Health Centre (Ontario Veterinary College, Guelph) for

Figure 9. The prevalence of deformities (%) in newly-transformed froglets from St. Clair River AOC locations and the two upstream reference sites, Wood Road and Port Franks, in 2006, 2007, 2011, and 2014. Numbers above bars represent the number of froglets examined and "*" indicates significant difference relative to pooled reference sites in the same year of comparison ($p<0.05$). The bold line indicates the 5% threshold for deformities which is considered elevated in wild populations of amphibians (Ouellett 2000).



assessment by Doug Campbell. Necropsies were performed on eight of these individuals and after histological assessment, all were found to contain parasitic trematodes. Specifically trematodes were present in a number of locations but the greatest numbers were in the legs where they likely were the cause of observed congestion and inflammation of the extremities. Further investigations of the parasites found in the feet and limbs of these frogs by David Marcogliese of Environment Canada suggest that this is a strigeid trematode, likely *Neodiplostomum* (which matures in raptors) or *Fibricola* (which matures in mammals), with frogs in either case serving as the intermediate host in its multi-host life cycle. The identification of the parasite(s) infecting St. Clair River AOC frogs was not confirmed.

In 2014, deformity frequencies in froglets were low ranging from 0% at Goose Lake, Chematogan Channel, and Bay Lodge to 2.6% at Dyke Road (Figure 9). A total of four deformed froglets were found at Bear Creek (2), Dyke Road (1), and Port Franks (1). Three of these frogs had deformities involving missing or partially-missing digits while the froglet from Port Franks had a missing front leg. No significant differences in the prevalence of deformities were found in froglets between each of the AOC locations and the Port Franks reference site.

Overall, 18 frog surveys were conducted at seven locations within the AOC over four study years. For 16 of these, deformity frequencies in newly-transformed froglets were below the 5% threshold for deformities which is considered elevated in wild populations of amphibians (Ouellett 2000). This threshold was exceeded in two surveys conducted at Chematogan Channel and Bay Lodge in 2007. It is noteworthy that subsequent surveys conducted at these two locations in 2011 and/or 2014 revealed a low prevalence of deformities in froglets that was well below this threshold. The threshold was not exceeded at either of the two reference sites in any study year.

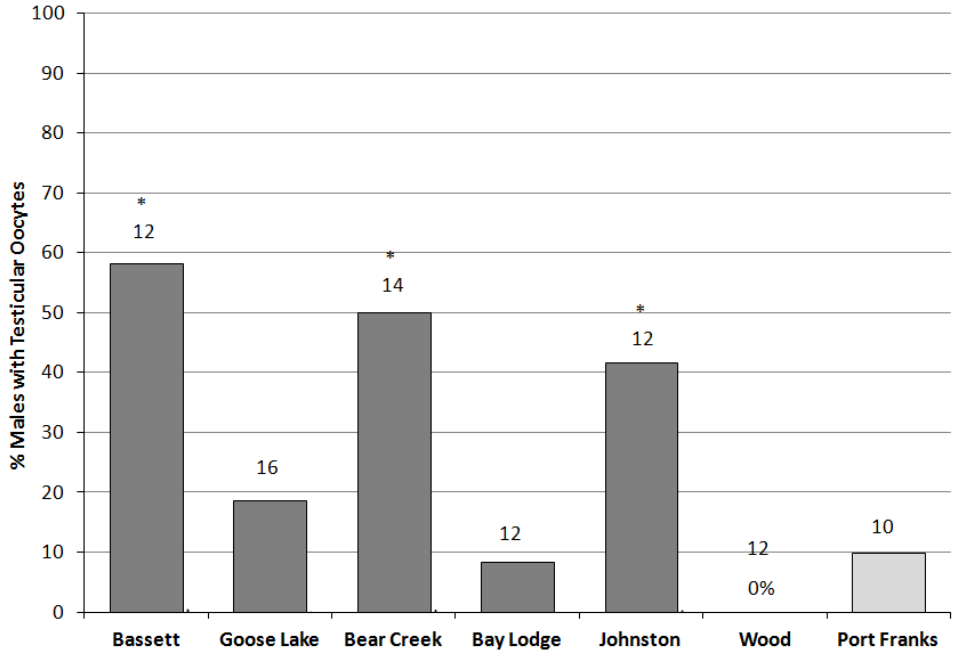
Gonadal Histology and Vitellogenin-like Protein

In 2006, histological examinations of testes in maturing pre-hibernation young-of-year frogs revealed the presence of testicular oocytes in male frogs from all AOC locations (Figure 10a). The prevalence of males with testicular ooctyes ranged from 8.3% at Bay Lodge to 58.3% at Bassett Channel. Frequencies of males with this condition at the two reference sites, Wood Road and Port Franks, were equal to 0% and 10.0%, respectively, and were not significantly different ($p>0.05$). Overall, proportions of males with testicular ooctyes were significantly higher in males from three AOC sites, Bassett Channel (Fisher exact test, $p=0.0010$), Bear Creek (Fisher exact test, $p=0.0026$) and Johnston Channel (Fisher exact test, $p=0.0136$), relative to the pooled reference sites.

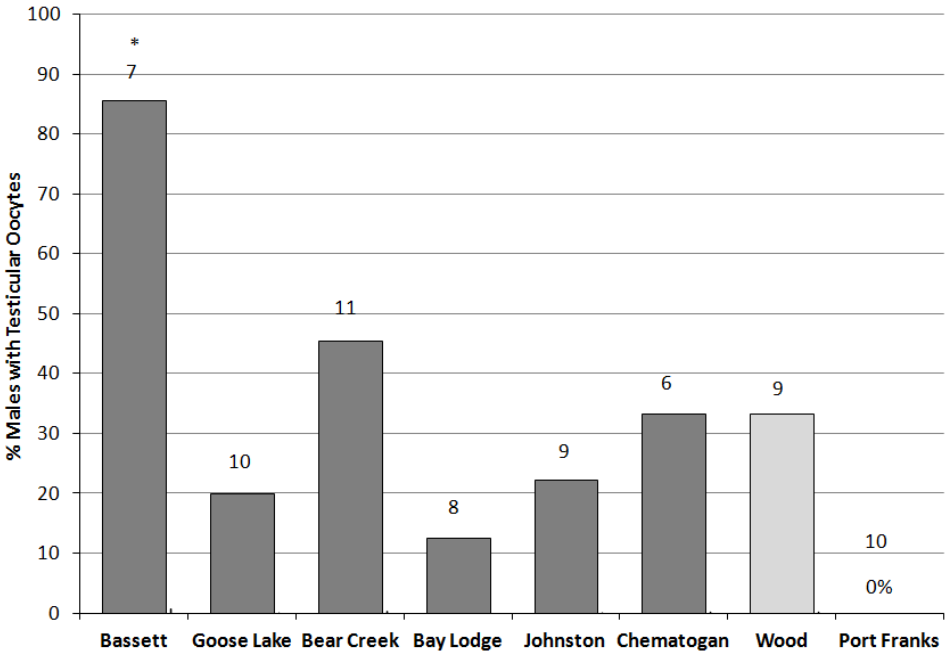
In 2007, the prevalence of males with testicular ooctyes at AOC locations ranged from 12.5% at Bay Lodge to 85.7% at Bassett Channel (Figure 10b). Frequencies of males with testicular ooctyes at the two reference sites were equal to 33.3% and 0% at Wood Road and Port Franks, respectively, and were not significantly different ($p>0.05$). Overall, the proportion of males with this condition was significantly higher at one AOC location only, Bassett Channel, compared to the pooled reference sites (Fisher exact test, $p=0.0022$).

Of the 41 male frogs examined from five St. Clair River AOC locations and 15 frogs from the two reference sites in 2006, no frogs tested positive for the vitellogenin-like protein. Similarly in 2007, of the 41 male frogs from six AOC locations and 15 frogs examined from the two reference sites, no frogs tested positive for this protein.

Figure 10. The prevalence (%) of male young-of-year northern leopard frogs with testicular oocytes from St. Clair River AOC locations and two upstream reference sites in 2006 (a) and 2007 (b). Numbers above bars indicate the number of male frogs examined and "*" indicates significant difference relative to pooled reference sites.



a) 2006



b) 2007

Contaminants in Northern Leopard Frogs

Significant spatial differences in mean concentrations of organochlorine compounds were evident in northern leopard frogs collected from the St. Clair River AOC and reference study sites in 2006 and 2007 (Table 2). Overall, there was a significant difference in mean sum PCB body burdens among sites ($H=26.49$, $p=0.0009$) with significantly higher PCB concentrations in frogs from Chematogan Channel versus those from Heritage-White Bread and the two upstream Lake Huron reference sites, Wood Road and Port Franks. Sum PCBs in frogs were low with means generally ranging between 1-2 ng/g at study sites and concentrations below 5.3 ng/g in all samples. One exception is the one pooled sample from Wood Road with a high sum PCB concentration (37.4 ng/g) which resulted in an elevated mean sum PCB concentration of 8.3 ng/g and notably high variability observed among frogs at that site. Overall PCB body burdens in frogs were well below the IJC Aquatic Life Guideline of 100 ng/g for the protection of fish-eating wildlife (IJC 1988).

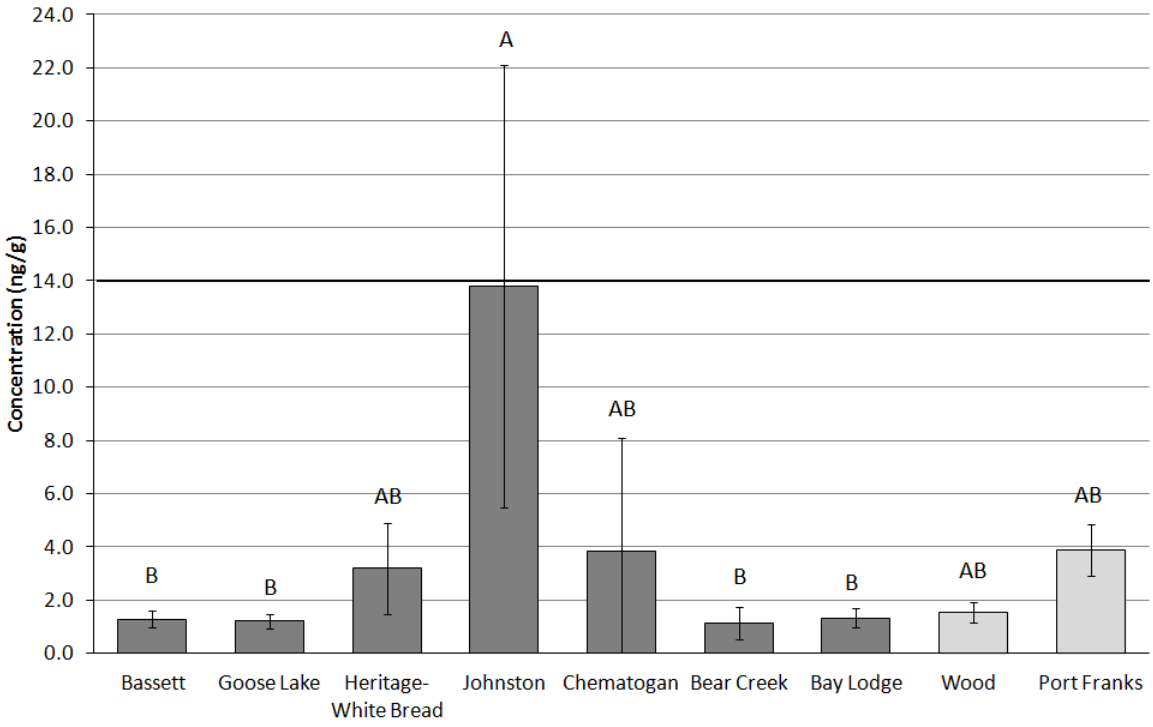
Total DDT concentrations, as the sum concentration of *p,p'*-DDE, *p,p'*-DDT and *p,p'*-DDD, were largely similar to sum PCB concentrations with means ranging from 1-4 ng/g at most study sites (Figure 11). Concentrations of total DDT in frogs were significantly different among sites ($H=30.55$, $p=0.0002$). Frogs from Johnston Channel had a significantly higher mean total DDT concentration than frogs from Bassett Channel, Goose Lake, Bear Creek, and Bay Lodge with frogs from the reference sites showing intermediate mean total DDT concentrations. Frogs from Johnston Channel also had a mean total DDT concentration of 13.8 ng/g which approached the Canadian Environmental Quality Guideline of 14.0 ng/g for the protection of wildlife consumers of aquatic biota (CCME 2001). Specifically, two of five pooled samples of frogs from Johnston Channel had total DDT concentrations (25.3 ng/g and 18.9 ng/g) which exceeded this guideline. One of five pooled frog samples from Chematogan Channel had an elevated total DDT concentration equal to 12.0 ng/g while frog samples from other sites had total DDT concentrations below 6.2 ng/g. Similar spatial patterns were evident for *p,p'*-DDE, as the primary metabolite of total DDT, and which comprised on average 91.8% of total DDT found in frogs in this study (Table 2).

Mean concentrations of the remaining organochlorine compounds in frogs from study sites were at most one-third of total DDT concentrations, generally below 1 ng/g and found below the limit of detection for some compounds at some sites (Table 2). Hexachlorobenzene (HCB) was found in pooled samples of frogs from all sites with means ($\pm$SD) ranging from 0.09 ($\pm$0.04) ng/g at Goose Lake to 0.40 ($\pm$0.53) ng/g at Heritage-White Bread where the highest HCB concentration of 1.35 ng/g was found in a sample. As a result of elevated concentrations of dieldrin in three pooled samples of frogs from Johnston Channel (range=2-8 ng/g), dieldrin concentrations were approximately ten times higher at this location relative to the other three AOC locations where it was found above the limit of detection. Dieldrin was non-detectable in frogs from three of seven AOC locations. Sum chlordane (as the sum concentration of oxychlordane, *cis*-and *trans*-chlordane and *cis*- and *trans*-nonachlor) was significantly higher in frogs from Chematogan Channel compared to concentrations in frogs from Wood Road and Bassett Channel. Three pooled samples of frogs from Port Franks had the highest sum chlordane concentrations in this study which ranged from 1.00-3.20 ng/g resulting in the highest mean concentration (1.25 ng/g) at that site. Similarly, one pooled sample from Port Franks had the highest

Table 2. Mean (SD) and maximum concentrations (ng/g wet weight) of organochlorine compounds and sum PBDEs in northern leopard frogs from seven St. Clair River AOC (including WIFN) locations and two upstream reference sites in 2006 and 2007. N denotes the number of pooled samples analyzed each consisting of two individuals of the same sex. ND denotes below the level of detection. Different uppercase letters represent sites which were significantly different from one another as determined using one-way parametric or non-parametric analysis of variance tests and post-hoc analyses. In some cases where a significant difference was detected among study sites, post-hoc analyses may not have revealed significant differences for individual site comparisons (e.g., dieldrin).

	St. Clair River Area of Concern							**Reference**		
	WIFN Locations					**Other Locations**				**Statistic**
Compound	**Bassett Channel**	**Goose Lake**	**Heritage-White Bread**	**Johnston Channel**	**Chematogan Channel**	**Bear Creek**	**Bay Lodge**	**Port Franks**	**Wood Rd.**	
Year	**2006**	**2006**	**2006**	**2006**	**2007**	**2007**	**2007**	**2006**	**2006**	
N	5	5	5	5	7	6	6	5	5	
Sum PCBs	1.97 (0.21) 2.13 **AB**	1.66 (0.22) 1.74 **AB**	1.22 (0.18) 1.47 **B**	1.54 (0.15) 1.72 **AB**	2.28 (0.41) 3.04 **A**	1.95 (1.63) 5.27 **AB**	1.85 (0.51) 2.79 **AB**	1.27 (0.18) 1.48 **B**	8.25 (16.28) 37.4 **B**	H=26.49 p=0.0009
p,*p*'-DDE	1.18 (0.31) 1.62 **BC**	1.11 (0.27) 1.51 **BC**	3.10 (1.71) 6.07 **ABC**	13.70 (8.31) 25.34 **A**	3.35 (3.83) 10.95 **ABC**	0.99 (0.60) 2.16 **C**	1.17 (0.27) 1.51 **BC**	3.80 (0.97) 4.71 **AB**	1.44 (0.37) 1.84 **ABC**	H=30.58 p=0.0002
HCB	0.26 (0.06) 0.31 **A**	0.09 (0.04) 0.17 **B**	0.40 (0.53) 1.35 **AB**	0.16 (0.04) 0.18 **AB**	0.20 (0.06) 0.26 **AB**	0.14 (0.03) 0.17 **AB**	0.19 (0.02) 0.21 **AB**	0.17 (0.07) 0.27 **AB**	0.27 (0.46) 1.10 **AB**	H=20.04 p=0.010
Dieldrin	ND	ND	ND	2.44 (3.19) 8.00	0.24 (0.09) 0.37	0.28 (0.32) 0.91	0.24 (0.10) 0.37	ND	ND	H=31.19 p=0.0001
Sum Chlordane	0.25 (0) 0.25 **B**	0.31 (0.15) 0.60 **AB**	0.27 (0.03) 0.32 **AB**	0.48 (0.31) 0.97 **AB**	0.54 (0.15) 0.76 **A**	0.33 (0.06) 0.43 **AB**	0.45 (0.03) 0.51 **AB**	1.25 (1.22) 3.20 **AB**	0.25 (0) 0.25 **B**	H=25.72 p=0.0012
Heptachlor Epoxide	ND	0.07 (0.05) 0.16	ND	0.08 (0.06) 0.18	0.07 (0.03) 0.13	ND	ND	0.26 (0.47) 1.09	ND	H=31.91 p=0.0001
OCS	0.41 (0.20) 0.63 **A**	ND **ABC**	ND **ABC**	0.19 (0.10) 0.28 **AB**	0.06 (0.02) 0.11 **C**	ND **C**	0.06 (0.02) 0.10 **BC**	ND **ABC**	ND **ABC**	H=37.80 p<0.0001
Sum PBDEs	0.43 (0.18) 0.73	0.20 (0.07) 0.30	0.18 (0.03) 0.23	0.21 (0.08) 0.34	-	-	-	0.15 (0.03) 0.19	0.31 (0.44) 1.10	F=2.33 p=0.07

Figure 11. Mean concentrations (SD, ng/g wet weight) of total DDT in northern leopard frogs from seven St. Clair River AOC locations and two upstream reference sites, Wood Road and Port Franks, in 2006 and 2007. The solid black line indicates the Canadian Environmental Quality Guideline associated with the protection of wildlife consumers of aquatic biota for total DDT (CCME 2001). Different uppercase letters represent sites which were significantly different from one another.



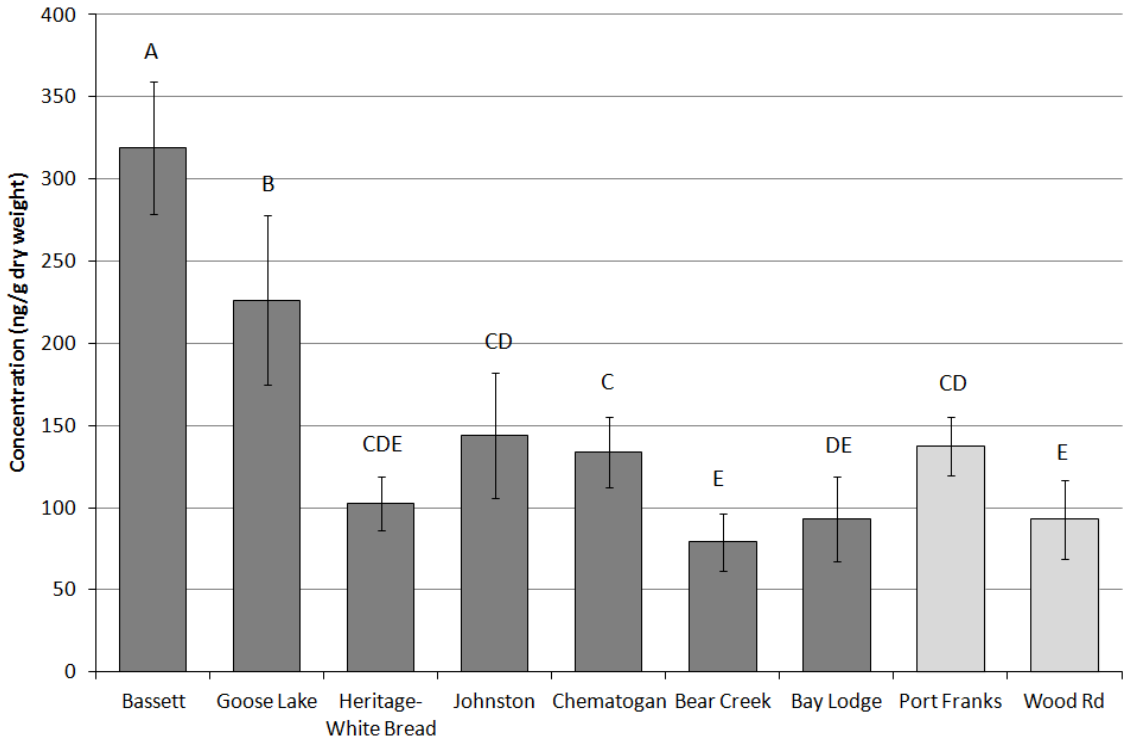
concentration of heptachlor epoxide (1.09 ng/g) which contributed to the highest mean concentration of HE found at that site (0.26 ng/g). In contrast, concentrations of sum chlordane, HE and dieldrin at the other reference site, Wood Road, were non-detectable. While also considered extremely low, relatively higher concentrations of octachlorostyrene (OCS) were found in frogs from Bassett Channel where the mean OCS concentration (0.41 ng/g) was significantly higher relative to frogs from Chematogan Channel, Bear Creek and Bay Lodge. Mirex was not detected in any samples.

Mean sum PBDE (±SD) concentrations ranged from 0.15 (±0.03) ng/g in frogs from Port Franks to 0.43 (±0.18) ng/g in frogs from Bassett Channel. The dominant congeners were BDE-47, BDE-99, BDE-100 and BDE-153 which contributed on average 96.7% (±0.35%) to the total sum PBDEs in frogs (range=93.8-100%). One additional congener, BDE-154/BB-153, was the only other congener found above the limit of detection and only at some sites. One frog sample from Wood Road had both the highest sum PBDE concentration (1.10 ng/g) and sum PCB concentration (37.4 ng/g) and incidentally was the only sample where a detectable concentration of hexabromocyclododecane (HBCDD, equal to 0.02 ng/g), another flame retardant, was found. BB-101 was found below the limit of detection in all samples. While sum PBDE concentrations in frogs were not significantly different among sites sampled in 2006 ($F_{5,24}$=2.33, p=0.07), they were found at higher concentrations relative to some organochlorine compounds and

above the limit of detection in all analyses (i.e., >0.1 ng/g). Chemical analyses for PBDEs were not performed in frogs collected in 2007.

Mean mercury (±SD, dry weights) concentrations in frogs from the seven St. Clair River AOC locations and two reference sites ranged from 78.98 (±17.47) ng/g in frogs from Bear Creek to 319.01 (±40.34) ng/g in frogs from Bassett Channel (Figure 12; range in mean concentrations on a wet weight basis from 18.20 (±4.03) ng/g to 72.01 (±16.14) ng/g)). Overall, mercury concentrations varied significantly among study sites with significantly higher mean concentrations in frogs from Bassett Channel and Goose Lake compared to mean concentrations found in frogs from the other study sites, including the two reference sites ($F_{8,78}$=39.07, p<0.00001).

Figure 12. Mean concentrations (SD, ng/g dry weight) of total mercury in whole northern leopard frogs from seven St. Clair River AOC locations and two upstream reference sites, Wood Road and Port Franks, in 2006 and 2007. Nine or ten individuals were analyzed per site. Different uppercase letters represent sites which were significantly different from one another.



Water Chemistry - Pesticides, Nutrients and Metals

For those herbicides detected at least once in water samples collected in 2006 and/or 2007, concentrations were low overall at all study sites and below the limit of detection in the majority of cases (72%; Table 3). There was no obvious spatial pattern of pesticides in water from AOC versus reference sites and, on occasion, concentrations of some compounds were higher in water from reference sites relative to AOC locations. Of the phenoxy-acid herbicides, 2,4-D (and its metabolite, 2,4-DP), clopyralid and dicamba were the most frequently detected compounds. In 2006, concentrations of

these compounds were generally highest at the upstream Lake Huron reference sites while, in 2007, concentrations were higher at non-WIFN AOC locations relative to the reference sites. This is in part due to the inclusion of five additional sampling locations along the St. Clair River and north of WIFN in 2007. MCPA was found at Bear Creek only in both 2006 and 2007 at very low concentrations (8.21 ng/L and 3.45 ng/L, respectively). Bromoxynil was detected in 2007 at Baby Creek (3.63 ng/L) and Bear Creek (4.76 ng/L) and) while Imazethapyr was found at the Port Franks reference site in both May and June of 2006 (1.63 ng/L and 11.3 ng/L, respectively). Mecoprop was detected at one WIFN location (Chematogan Channel, 39.9 ng/L), two non-WIFN AOC locations (Bay Lodge, 3.58 ng/L and Bear Creek, 1.69 ng/L) and both reference sites (Port Franks, 2.34 ng/L and Wood Road, 13.4 ng/L). Phenoxy-acid herbicide concentrations never exceeded Canadian Water Quality Guidelines (CWQGs) for the protection of aquatic life (CCME 2007). Other phenoxy-acid herbicides including 2,3,6-TBA, Silvex, 2,4,5-T, MCPB, 2,4-DB, Picloram and Imazamethabenz-methyl(A) and (B) were not detected at any sites. A complete listing of concentrations of the phenoxy-acid and neutral herbicides at all sites for each of the three sampling periods (by month, where data are available) is provided in Appendix 3.

Of the neutral herbicides, atrazine, its breakdown product D-atrazine, and metolachlor were the most frequently detected compounds (Table 3). Atrazine was generally found at the highest concentrations of all pesticides with the highest reported concentration of 1,610 ng/L at Port Franks in 2006 which approached the CWQG of 1,800 ng/L for the protection of aquatic life (CCME 2007). In addition, atrazine was the only pesticide which was found above the limit of detection in every water sample collected from every site. At six of eight study sites where atrazine was measured in both May and June/July (i.e., Bassett Channel, Goose Lake, Bear Creek, Terra, Port Franks, and Wood Road (2006)), concentrations were higher in June/July relative to May (Appendix 3). Metolachlor concentrations were also high relative to other pesticides with the two highest concentrations found at Port Franks (equal to 156 ng/L and 906 ng/L in May and June 2006, respectively) but which were well below CWQGs. Both simazine and metribuzin were detected in water from Port Franks in May 2006 (28 ng/L and 894 ng/L, respectively). Water from Goose Lake and Chematogan Channel were the two WIFN locations which most frequently had the maximum concentrations of herbicides in 2006 and 2007, respectively, while of the non-WIFN AOC locations, Bear Creek most frequently had the highest concentrations in both study years (Appendix 3). Maximum herbicide concentrations were more frequently found in water from Port Franks relative to Wood Road in both study years. Of the remaining neutral herbicides analyzed, butylate, desethylsimazine, trifluralin, diallate-1, diallate-2, triallate, dichlofopmethyl (hoegrass), and benzoylprop-ethyl (Endaven) were not detected at any sites. Organophosphate pesticides were not detected in water samples collected in 2006.

Table 3. Concentrations of phenoxy-acid and neutral herbicides in water (ng/L) from several St. Clair River AOC (including WIFN) locations and upstream reference sites in 2006 and 2007. Concentrations are shown as median (maximum) and number of collections where compound was found above the limit of detection/total number of collections. Mean (maximum) concentrations of nutrients (mg/L) are also shown (where nitrogenous compounds are expressed as mg/L of N). Canadian Water Quality Guidelines (CWQGs) for the protection of aquatic life are also indicated for each compound (CCME 2007). ND indicates pesticide concentration was below the limit of detection*.

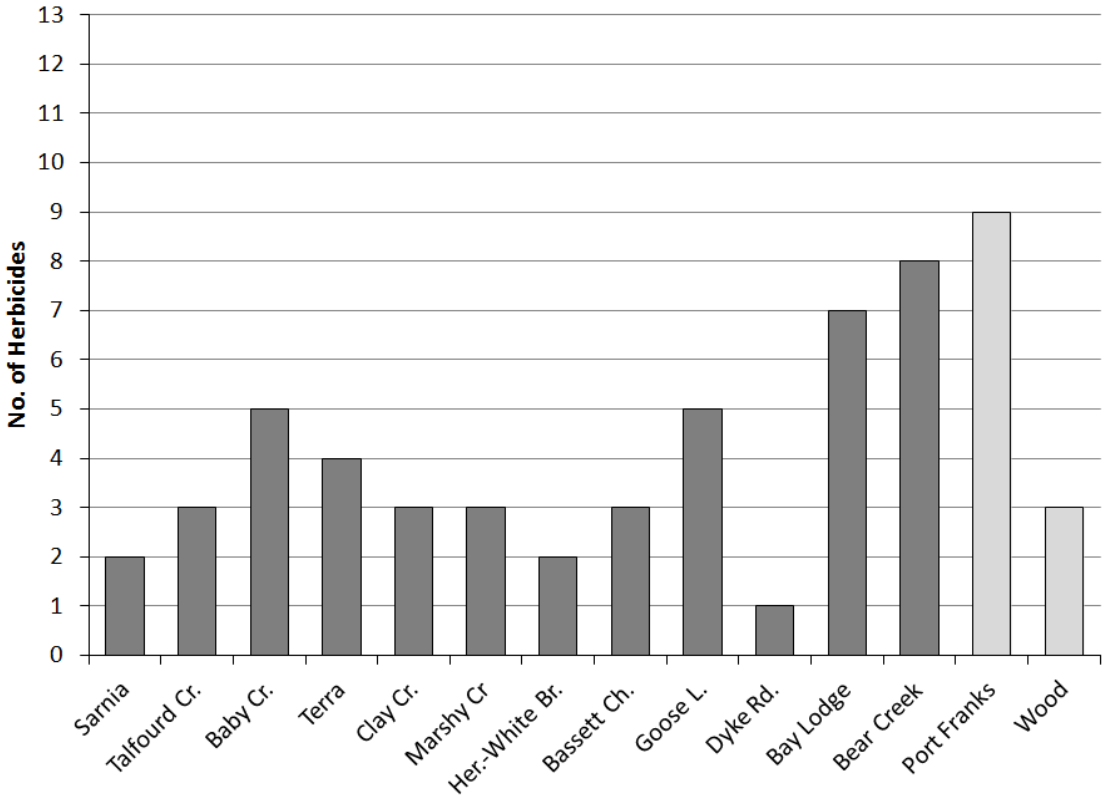
	CWQG	Year	St. Clair River Area of Concern		Reference Sites
Compound			**WIFN** ** **Locations**	**Other AOC Locations**	
Clopyralid	4,000	2006	0.30 (1.64) 3/9	ND 0/4	1.45 (10.9) 4/6
		2007	0.30 (3.83) 1/7	0.30 (5.73) 2/11	ND 0/5
Dicamba	10,000	2006	0.37 (2.43) 2/9	0.37 (4.15) 1/4	1.31 (744) 3/6
		2007	ND 0/7	0.37 (103) 5/11	0.37 (15.4) 2/5
2,4-D	4,000	2006	3.12 (24.0) 8/9	5.5 (95) 3/4	13.0 (82.8) 6/6
		2007	0.24 (27.3) 2/7	5.1 (157) 8/11	0.24 (7.62) 1/5
2,4-DP	4,000	2006	0.21 (2.83) 3/9	0.99 (16.4) 3/4	1.70 (3.66) 4/6
		2007	ND 0/7	0.21 (5.35) 5/11	ND 0/5
MCPA	2,600	2006	ND 0/9	0.29 (8.21) 1/4	ND 0/6
		2007	ND 0/7	0.29 (3.45) 1/11	ND 0/5
Bromoxynil	5,000	2006	ND 0/9	ND 0/4	ND 0/6
		2007	ND 0/7	0.50 (4.76) 2/11	ND 0/5
Mecoprop	4,000	2006	ND 0/9	0.25 (1.69) 1/4	0.25 (13.4) 2/6
		2007	0.25 (39.9) 1/7	0.25 (3.58) 1/11	ND 0/5
Imazethapyr	NA	2006	ND 0/9	ND 0/4	0.60 (11.3) 2/6
		2007	ND 0/7	ND 0/11	ND 0/5
Atrazine	1,800	2006	25.8 (86.9) 8/8	40.0 (98.5) 4/4	84.0 (1,610) 6/6
		2007	16.7 (51.6) 7/7	31.9 (378) 11/11	16.8 (161) 5/5
D-atrazine	1,800	2006	13.4 (51.5) 3/8	38.0 (57.8) 3/4	40.4 (322) 4/6
		2007	13.4 (42.9) 1/7	13.4 (133) 5/11	13.4 (42.5) 1/5
Metolachlor	7,800	2006	11.9 (70.9) 3/8	53.2 (128) 2/4	25.7 (906) 3/6
		2007	ND 0/7	11.9 (173) 4/11	ND 0/5
Simazine	10,000	2006	ND 0/8	ND 0/4	8.2 (28) 1/6
		2007	ND 0/7	ND 0/11	ND 0/5
Metribuzin	1,000	2006	ND 0/8	ND 0/4	10.4 (894) 1/6
		2007	ND 0/7	ND 0/11	ND 0/5
Nitrates	13	2006	0.19 (0.29) 6/6	0.80 (1.92) 3/3	1.35 (4.81) 4/4
		2007	0.13 (0.36) 3/3	1.74 (8.29) 9/9	0.04 (0.10) 3/3
Ammonia	1.37	2006	0.058 (0.080) 6/6	0.11 (0.18) 3/3	0.058 (0.12) 4/4
		2007	0.043 (0.088) 3/3	0.045 (0.12) 9/9	0.090 (0.19) 3/3
Phosphate	NA	2006	0.009 (0.018) 9/9	0.029 (0.042) 4/4	0.034 (0.072) 6/6
		2007	0.047 (0.097) 3/3	0.033 (0.069) 9/9	0.033 (0.054) 3/3

* Detection limits for pesticides were as follows: clopyralid (0.59 ng/L), dicamba (0.73 ng/L), 2,4-D (0.47 ng/L), 2,4-DP (0.42 ng/L), MCPA (0.58ng/L), bromoxynil (0.99 ng/L), Mecoprop (0.50 ng/L), Imazethapyr (1.20 ng/L), atrazine (1.86 ng/L), D-atrazine (26.8 ng/L), metolachlor (23.70 ng/L), simazine (16.4 ng/L), and metribuzin (20.7 ng/L).
** Includes White Bread sampling location in 2007

Given the high frequency of water samples with herbicide concentrations found below the limit of detection, another way of describing the data is to tally and compare the number of herbicides which were found above the detection limit at study sites. This approach is useful for visualizing the number of

water-borne compounds which frogs may be exposed to during a particular period in their development. In this study, May is the preferable month for comparison since water was most consistently collected from 14 of 16 possible study sites. Figure 13 shows the number of pesticides found above the detection limit out of a possible 13 herbicides which were detected at least once during this period; an average was calculated if water was sampled from a site in both 2006 and 2007. Water from Port Franks had the greatest number of compounds detected in May of 2006 where nine of 13 compounds were found above the detection limit. In contrast, only one or two compounds were detected in May from water from three AOC sites, Dyke Road, Sarnia and Heritage-White Bread. Water from the remaining sites had intermediate numbers of compounds found above the detection limit.

Figure 13. Number of detectable herbicides (of 13 possible compounds) in water samples collected from 12 St. Clair River AOC locations and two upstream reference sites, Wood Road and Port Franks, in May of 2006 and/or 2007.



Nutrient concentrations were generally low and below 2 mg/L at all sites with the exception of nitrate concentrations at Bear Creek in May 2007 (8.29 mg/L) and Port Franks in June 2006 (4.81 mg/L) which were elevated but below CWQG concentrations for the protection of aquatic life (CCME 2007; Table 3). At the three sites where nitrate concentrations were measured in both May and June, Bay Lodge, Bear Creek and Wood Road, concentrations were higher in May relative to June (Appendix 3).

Trace metals were high in some water samples from AOC and reference sites occasionally exceeding CWQGs for the protection of aquatic life (CCME 2007; Table 4). Aluminum most frequently exceeded its

Table 4. Trace metal concentrations (µg/L) in water collected from several St. Clair River AOC (including WIFN) locations and upstream reference sites in May and June of 2006 and 2007 where data are available. Canadian Water Quality Guidelines (CWQGs) for the protection of aquatic life are indicated for each compound (CCME 2007). Concentrations in bold indicate those samples that exceeded CWQGs.

		St. Clair River Area of Concern													**Reference Sites**	
		WIFN Locations						**Other Locations**								
Compound	**CWQG**	**Bassett Channel**	**Goose Lake**	**Heritage-White Bread**		**Terra**	**Sarnia**	**Talfourd Creek**	**Baby Creek**	**Clay Creek**	**Marshy Creek**	**Bear Creek**	**Bay Lodge**	**Port Franks**	**Wood Rd.**	
Year		**2006**	**2006**	**2006**	**2007**	**2006**	**2007**	**2007**	**2007**	**2007**	**2007**	**2007**	**2007**	**2006**	**2006**	**2007**
Aluminum	100															
May		**375**	**453**	45.9	45.1	**1120**	36.1	**161**	**748**	**784**	71.0	**961**	**217**	**468**	**1210**	**586**
June		**157**	44.5	55.9	-	75.3	-	-	-	-	-	-	-	**306**	**2600**	-
Arsenic	5.0															
May		0.79	0.65	0.47	0.47	1.10	0.44	1.30	1.17	0.79	0.50	0.84	0.73	0.65	1.34	1.53
June		0.76	0.95	0.48	-	0.56	-	-	-	-	-	-	-	0.82	2.17	-
Cadmium	0.017															
May		**0.023**	**0.053**	0.009	0.004	**0.037**	0.005	**0.019**	**0.044**	**0.024**	0.007	**0.024**	0.010	0.014	**0.025**	0.013
June		0.012	0.012	0.011	-	0.009	-	-	-	-	-	-	-	0.013	**0.041**	-
Chromium	1															
May		0.762	0.938	0.195	0.201	**2.08**	0.167	0.383	**1.21**	**1.27**	0.237	**1.32**	0.374	0.764	**2.04**	**1.00**
June		0.367	0.151	0.240	-	0.280	-	-	-	-	-	-	-	0.496	**4.34**	-
Copper	2															
May		1.52	**2.08**	0.66	0.81	**3.97**	0.64	**6.62**	**2.96**	**2.66**	0.90	**2.97**	1.31	**2.12**	**2.53**	1.74
June		0.90	0.85	0.71	-	1.26	-	-	-	-	-	-	-	**2.66**	**3.94**	-
Iron	300															
May		**721**	**905**	67.5	60.4	**1970**	33.3	240	**1070**	**1060**	101	**1360**	**349**	**684**	**2310**	**1130**
June		**324**	154	85.2	-	142	-	-	-	-	-	-	-	**445**	**4610**	-
Lead	2															
May		0.92	**2.31**	0.07	0.06	1.60	0.04	0.29	0.75	0.74	0.10	1.01	0.31	0.46	1.43	0.79
June		0.39	0.40	0.23	-	0.08	-	-	-	-	-	-	-	0.30	**2.68**	-
Moly-bdenum	73															
May		0.62	1.65	0.54	0.55	1.00	0.56	1.33	4.47	1.30	0.57	2.00	1.22	0.82	2.75	3.28
June		0.59	0.84	0.55	-	0.81	-	-	-	-	-	-	-	1.12	3.27	-
Nickel	65															
May		1.45	1.77	0.59	0.50	3.42	0.48	1.42	2.63	2.33	0.58	2.14	1.04	1.73	5.23	3.15
June		0.77	0.53	0.50	-	0.58	-	-	-	-	-	-	-	1.48	0.50	-
Selenium	1.0															
May		0.18	0.17	0.14	0.14	0.14	0.09	0.30	0.77	0.29	0.13	**1.53**	0.58	0.26	0.17	0.19
June		0.15	0.17	0.18	-	0.19	-	-	-	-	-	-	-	0.29	0.20	-
Silver	0.1															
May		0.006	0.006	0.003	0.006	0.011	0.003	0.004	0.011	0.011	0.009	0.010	<0.001	0.003	0.012	0.004

		St. Clair River Area of Concern												Reference Sites	
		WIFN Locations				Other Locations									
Compound	**CWQG**	**Bassett Channel**	**Goose Lake**	**Heritage-White Bread**		**Terra**	**Sarnia**	**Talfourd Creek**	**Baby Creek**	**Clay Creek**	**Marshy Creek**	**Bear Creek**	**Bay Lodge**	**Port Franks**	**Wood Rd.**	
Year		**2006**	**2006**	**2006**	**2007**	**2006**	**2007**	**2007**	**2007**	**2007**	**2007**	**2007**	**2007**	**2006**	**2006**	**2007**
June		0.003	0.001	0.001	-	0.002	-	-	-	-	-	-	-	0.003	0.012	-
Thalium	0.8															
May		0.011	0.010	0.004	0.004	0.025	0.004	0.008	0.028	0.015	0.005	0.012	0.003	0.008	0.035	0.014
June		0.007	0.003	0.005	-	0.005	-	-	-	-	-	-	-	0.007	0.054	-
Zinc	30															
May		2.94	5.76	1.26	1.04	7.94	0.70	5.36	6.46	4.56	0.92	6.50	1.69	3.36	6.82	4.09
June		1.10	1.21	0.55	-	0.78	-	-	-	-	-	-	-	1.75	12.9	-

respective guideline (67% of water samples) followed by iron (62%) cadmium (48%), copper (45%), chromium (33%), lead (10%) and selenium (5%) while arsenic, molybdenum, nickel, silver, thalium and zinc were below CWQGs at all sites. Metal concentrations were most frequently highest in water from Bear Creek (where 46% or 6 of 13 water samples exceeded respective guidelines), followed by Wood Road in 2006 (42%), Baby Creek and Clay Creek (38% each) and Talfourd Creek, Port Franks and Wood Road in 2007 (23% each). The lowest concentrations of trace metals were generally found in water from the four WIFN locations where the fewest number of CWQG exceedences were also found (i.e., in less than 19% of water samples).

Discussion

Embryonic Exposure Studies

High hatchability (>98%) and low deformity frequencies (<2%) in northern leopard frog embryos raised in water from eight St. Clair River AOC locations in 2007 are consistent with background frequencies expected in wild populations and suggest that water from the St. Clair River does not pose a threat to amphibian hatching success or embryonic deformities. Since sediment can be a significant source of contaminants to biota in the aquatic environment, the study was repeated in 2011 using both water and sediment sampled from known sites of contamination in order to evaluate the impact of water and sediment exposure on embryonic development. Similar to the water exposure study, hatching success was high at five AOC locations (>98%) and embryonic deformity frequencies were low to moderate (<7%) with no significant site effect found. However if embryos had access to sediment in this experiment, it is likely that an increased incidence of embryonic deformities would have been found since sediment quality guidelines were exceeded for some compounds and most notably for mercury at all AOC locations. In the design of this experiment, individuals were physically separated from the sediments in order to prevent deaths from hypoxia during the egg stage. In the wild, embryos and tadpoles regularly come into contact with the sediment during normal foraging activities and filter feed sediment particles in the water column. In the case of leopard frogs, tadpoles spend 8-10 weeks between hatch and metamorphosis foraging at the sediment-water interface. Amphibians can accumulate appreciable amounts of organic contaminants, including highly lipophilic contaminants such as PCBs, through dermal exposure only (Johnson *et al.* 1999). Thus dermal and dietary exposure to contaminants can increase body burdens which could potentially increase the risk of deformities during critical periods of development. Savage *et al.* (2002) reported sublethal effects and increased mortality in wood frog (*Rana sylvatica*) tadpoles which had direct contact with sediment containing high concentrations of PCBs compared to those suspended above the sediment. In addition, if embryos were given an increased period of exposure to water and sediment (i.e. greater than one week), a higher incidence of deformities might have been evident particularly at the mouth of Bassett Channel where the concentrations of mercury and numerous PAHs in sediment were highest and many contaminants exceeded sediment quality guidelines associated with protection of aquatic life.

Embryonic deformities were broken down into two categories, impactful and low impact, based on potential impacts on survival and reproductive success in this study. Impactful deformities included stunted growth, edema, tail flexure, and missing or incomplete eyes. Embryos exposed to sediment and

water from the mouth of Bassett Channel had the greatest number of impactful deformities (Appendix 2) which could potentially adversely affect their survival or reproductive success by influencing, for example, predation rates, mating success and clutch size. Low impact deformities included gut formation and asymmetry (since the asymmetry was observed only in the placement of cement glands), either or both of which were evident in embryos from all study sites.

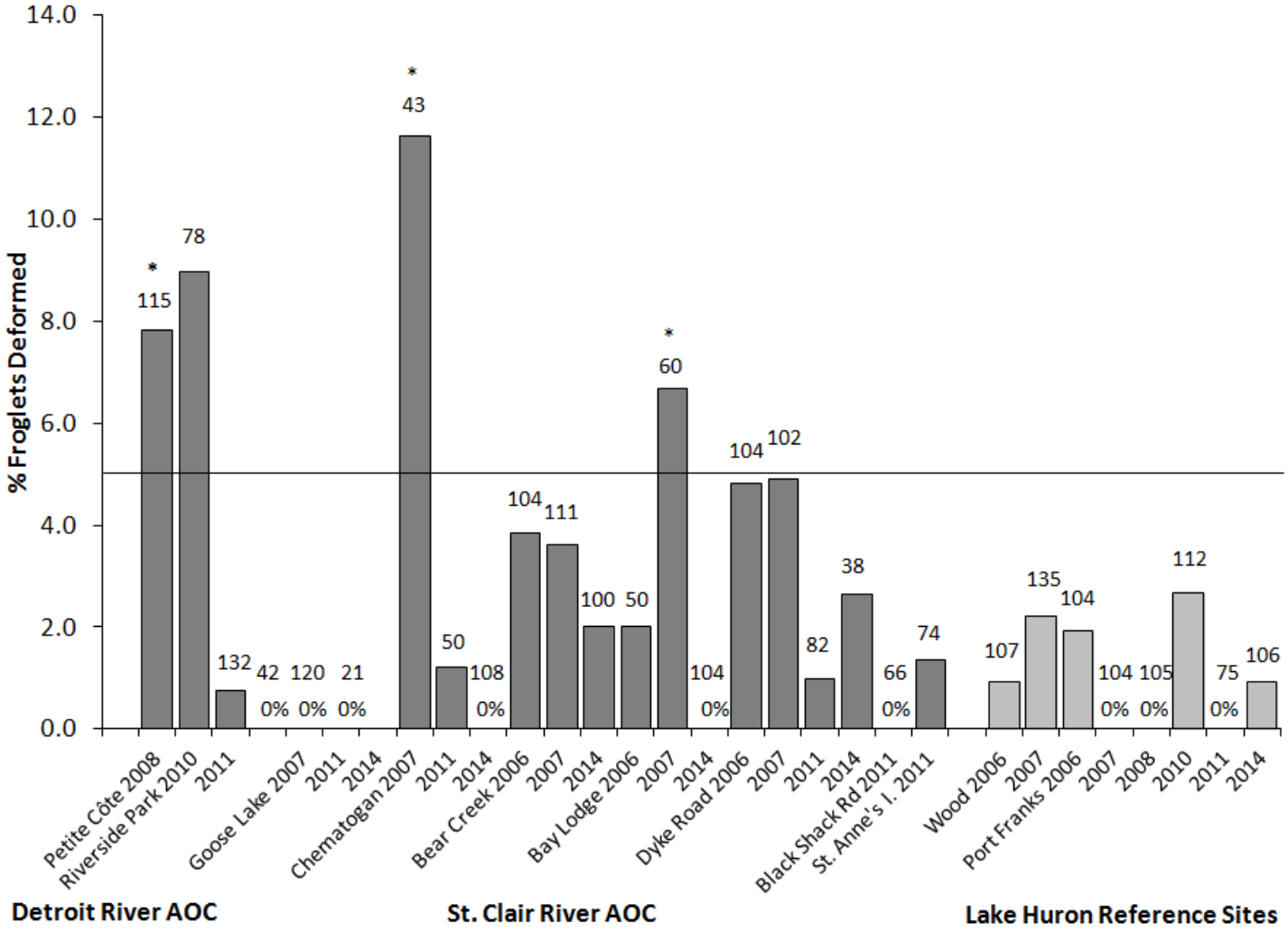
Deformities in Wild Populations of Newly-Transformed Froglets

At seven AOC locations, deformity frequencies in newly-transformed froglets ranged widely in the four study years (0%-11.6%) and significant site effects were evident in only one of four years (2007). Deformity frequencies at two AOC locations (Chematogan Channel and Bay Lodge) in 2007 were significantly higher compared to the reference sites where they also exceeded the 5% threshold which is considered elevated in wild amphibian populations (Ouellet 2000). The elevated deformity frequency in froglets from Chematogan Channel in 2007 (11.6%) was not evident in subsequent surveys conducted in 2011 (1.2%) or in 2014 (0%) when greater numbers of frogs were examined. Similarly, the elevated deformity frequency found at Bay Lodge in 2007 (6.7%) was not found either in 2006 (2.0%) or in 2014 (0%) when a greater number of froglets were surveyed. For the remaining AOC locations, deformity frequencies in froglets were below the 5% threshold.

Relative to other Great Lakes locations, deformity frequencies in froglets from St. Clair River AOC locations were lower (mean=2.5%) than frequencies found in froglets from Detroit River AOC locations (mean=5.9%) but higher than frequencies at upstream Lake Huron reference sites in 2006-2014 (mean=1.0%; Hughes *et al.* 2014a; Figure 14). Deformity frequencies in St. Clair River AOC froglets were also lower than frequencies reported in froglets from two St. Lawrence River AOC locations in 1998 and 1999 (mean=3.7%; McDaniel *et al.* 2004). Deformity frequencies in froglets from the St. Clair River AOC were low overall and likely within the expected range for wild amphibian populations. Notable variability in frequencies observed among years may be due, in some cases, to low numbers of frogs collected in some years (i.e., <100 frogs) and/or some variability in exposure attributable to unknown local stressors within the AOC.

Parasitic infection has been associated with limb deformities in amphibians (see Johnson *et al.* 2010 for review). The well-studied trematode *Ribeiroia* causes severe limb malformations in amphibians as a result of parasitic larvae which encyst around the developing limbs of amphibians resulting in severe disruptions in limb growth. High exposures to *Ribeiroia* increase both the risk and severity of malformations produced in larval amphibians as well as the likelihood that animals die following exposure. Stressors such as pesticides and eutrophication can increase rates of *Ribeiroia* infection by decreasing the ability of amphibians to elicit an immune response to prevent infection thus resulting in higher parasite loads and increased risk of limb deformities and mortality (Kiesecker 2002; Johnson *et al.* 2007). In this study, symptoms associated with possible trematode infection (i.e., red and swollen feet and/or legs) were evident in 2.0%-10.8% of frogs from three of five AOC locations in 2011. Similar symptoms were also reported in 24.2% of frogs from Riverside Park in the Detroit River AOC in 2011 where further investigation revealed evidence of parasitic infection by a strigeid trematode, likely

Figure 14. The prevalence of deformities in newly-transformed froglets from locations in the St. Clair River AOC, Detroit River AOC and upstream Lake Huron reference sites in 2006-2014. Numbers above bars indicate the number of frogs examined and "*" indicates significant difference relative to pooled reference sites in the corresponding year ($p<0.05$). The bold line indicates the 5% threshold identified for deformities which is considered elevated in wild populations of amphibians (Ouellett 2000).



Neodiplostomum (D. Marcogliese, EC, pers. comm.). In 2008, similar symptoms were reported in 95% of frogs from the Lake Huron Wood Road reference site where frogs were infected by unidentified immature larval trematode parasites in the connective tissue of the feet and where severe deformities were also found (EC unpublished data; D. Campbell, CCWHC, pers. comm.). Differences in the prevalence of apparent infection among sites suggest differences in intensity and/or timing of infection. It is unclear how the larval trematodes identified in the hind limbs and feet in frogs from Riverside Park and potentially in St. Clair River AOC frogs might adversely impact the long-term survival of leopard frogs. Further studies of the identity of these parasites and the effects of parasitic infection on frogs are necessary. If stressors such as pesticides and eutrophication also influence infection rates of the trematode identified in this study (and potentially development and survival of these frogs), the issue becomes increasingly complex and highlights the importance of considering parasitism when evaluating the influence of anthropogenic disturbance on amphibian declines and environmental health (Marcogliese *et al.* 2009).

Other Potential Effects

Endocrine-disrupting compounds including industrial chemicals such as PCBs and pharmaceuticals can also influence sexual differentiation by altering sex ratio and/or inducing an intersex condition in amphibians (Reeder *et al.* 1998; Mackenzie *et al.* 2003; Langlois *et al.* 2010). Sowers *et al.* (2009) demonstrated that larval exposure to municipal wastewater effluent resulted in an increased incidence of testicular oocytes, delayed metamorphosis and altered thyroid gland morphology in northern leopard frog juveniles. The prevalence of testicular ooctyes in male young-of-year leopard frogs from six St. Clair River AOC locations in 2006 and 2007 (mean=36%, range=8-86%) is intermediate to frequencies reported in this species from other southern Ontario agricultural sites in 2003-2005 (mean=42%, range=16-60%; McDaniel *et al.* 2008) and six Detroit River AOC locations in 2008 (mean=23%, range=0-83% using similar histological techniques; Hughes *et al.* 2014a). Gondal intersex has also been observed in white perch (*Morone americana*) from Lake St. Clair which drains the St. Clair River and where 45% of males had testicular ooctyes (Kavanagh *et al.* 2004). Over 58% of male frogs from Bassett Channel had testicular oocytes in 2006 and 2007 compared to male frogs from the two upstream reference sites in these two years where the prevalence was relatively lower (mean=11%, range=0-33%). In addition, significantly higher proportions of males from this AOC location had this condition compared to frogs from reference sites. The consistency of this finding in two study years is suggestive of exposure to an environmental stressor in this area. Spatial differences in observed frequencies may be a result of anthropogenic sources of environmental contaminants and/or pharmaceuticals originating in the St. Clair River AOC. However, elevated frequencies of males with testicular ooctyes at some AOC locations in this study were within ranges reported outside of the AOC and, as such, cannot be attributed to stressors within the AOC only. The implications of testicular oocytes in frogs in terms of reproduction or population viability are unknown. In a previous study on exposures of leopard frog tadpoles to agricultural run-off in the Thames River watershed, it was found that males from one site with significantly greater testicular oocyte sizes also exhibited significantly reduced sperm viability and motility (EC unpublished data).

Vitellogenin, a protein normally produced by females for egg production, has been used as a biomarker for exposure to environmental estrogens in amphibians and fish (Palmer *et al.* 1998; Kavanagh *et al.* 2004). Vitellogenin was detected in plasma of 8 of 10 male redhorse suckers (*Moxostoma macrolepidotum*) collected downstream of a wastewater treatment plant in the St. Clair River (Al-Ansari *et al.* 2010). This protein was not detected in male fish from the reference site situated approximately 26 kilometres further downstream at Port Lambton. The Port Lambton reference site in that study was relatively closer in proximity to AOC survey locations of frogs in this study and where this protein was also not found in males.

Atrazine, a triazine herbicide, used extensively in agricultural production, has been implicated in disrupting sexual differentiation both in the laboratory and in wild populations of northern leopard frogs (Hayes *et al.* 2003; McDaniel *et al.* 2008). Other studies have found no consistent relationship between atrazine in water and gonadal abnormalities observed in this and other ranid species (Coady *et al.* 2004; Orton *et al.* 2006; Murphy *et al.* 2006). In this study, no clear association was found between atrazine concentrations in water which varied between AOC and reference sites and the prevalence of testicular ooctyes in male frogs from study sites. Atrazine concentrations were low overall at study sites where water was sampled from creek outlets and marshes (Table 3, range in medians=17-84 ng/L) in contrast to water collected from sites adjacent to agricultural fields where atrazine concentrations are typically much higher (Murphy *et al.* 2006: range=170-250,000 ng/L; McDaniel *et al.* 2008: range in medians=68-780 ng/L). In addition, while reference sites were upstream of the AOC, these sites were not remote from agricultural influences. Specifically, both AOC and reference sites are located in Lambton County where relatively high herbicide use, including atrazine, was reported in 2008 (nearly 292 tonnes; McGee *et al.* 2010). This study has demonstrated that breeding frogs collected downstream of agricultural sites are exposed to a wide array of environmental contaminants, including numerous pesticides, nutrients as well as metals. These compounds have the potential to act synergistically, additively, or antagonistically (e.g., Hayes *et al.* 2006). Some studies have also found associations between the number of pesticides present in water and changes in survival and growth and increased frequencies of testicular ooctyes in amphibians (Relyea 2004; McDaniel *et al.* 2008) and well as changes in thyroid hormones in tree swallows (*Tachycineta bicolor*; Mayne *et al.* 2005). While concentrations of herbicides were overall low in water, there was some variability evident in the number of pesticides found above the detection limit among study sites in May. As demonstrated here, concentrations of pesticides may also fluctuate temporally over the course of the breeding season thereby influencing exposure during sensitive periods of egg and larval development as well as chronically over the life span of adults. All of these factors contribute to the difficulty in linking reproductive and developmental effects with exposure to specific compounds in wild populations.

Other developmental effects associated with elevated atrazine exposure in amphibians include gross morphological deformities, altered sex ratio, altered time to metamorphosis, altered plasma steroid concentrations and aromatase activity, reduction in laryngeal dilator muscle diameter, altered immune function as well as toxicity in eggs, larvae and metamorphs in the laboratory (see Bishop *et al.* 2010 for a review). With respect to atrazine concentrations associated with potential toxicological effects, concentrations in water were low overall and well below the concentration associated with acute

toxicity of late stage *R. pipiens* larvae (96 hours median lethal concentration LC50, equal to 14,500 ng/L; Howe *et al.* 1998). In addition, few water samples (i.e., 8 of 41 samples or 20%) had atrazine concentrations above the threshold of 100 ng/L associated with induction of gonadal abnormalities, testicular oocytes and hermaphroditism in leopard frogs and the African clawed frog (*Xenopus laevis*; Hayes *et al.* 2002, 2003). Conversely, Carr *et al.* (2003) reported that the incidence of gonadal abnormalities in *X. laevis* was only statistically significant from controls at 25,000 ng atrazine/L, a concentration several orders of magnitude higher than concentrations reported in this study. While no evidence of testicular ooctytes were found in atrazine-exposed individuals in a study conducted by Langlois *et al.* (2010), they found that leopard frog tadpoles exposed to 1,800 ng/L atrazine resulted in a sex-biased ratio in which 20% more females were produced compared to the control. One water sample from the Port Franks reference site (1,610 ng/L) approached this concentration which is also equal to the CWQG for the protection of aquatic life for atrazine. While atrazine was found most consistently and at the highest concentrations of all pesticides detected in water, concentrations were generally low at study sites and not likely sufficiently elevated to elicit effects on gonadal differentiation in male frogs.

Amphibians are sensitive to the toxic effects of metals in the environment (Linder and Grillitsch 2000). At sufficiently high exposures, aluminum has been associated with mortality in northern leopard frogs and laboratory toxicity studies have demonstrated that LC50s for embryos exposed to aluminum for 96 hours ranged from 400 to 1000 µg/L (Freda and McDonald 1990). Concentrations in water from most AOC locations and both reference sites were within this range where they also exceeded CWQGs for the protection of aquatic life (Table 4). Significant effects on embryonic survival or development however were not evident in the exposure study. Aluminum toxicity is complex and is influenced by water hardness, water pH and dissolved organic carbon which likely differed among sites. Concentrations of other metals, such as cadmium and lead, which exceeded CWQGs were likely below lowest effect concentrations associated with amphibian toxicity (Linder and Grillitsch 2000). However, sublethal effects associated with guideline exceedences in amphibians are unknown.

Contaminants

Effects of PCB exposure in northern leopard frogs include reduced hatching success, increased mortality and effects on development and metamorphosis (Rosenshield *et al.* 1999). In this study, concentrations of sum PCBs in frogs were generally low (i.e., below 3 ng/g) and were well below concentrations found in leopard frogs from known contaminated ecosystems. These include polluted sites in the Fox River and Green Bay watershed where PCBs in whole bodies of juvenile leopard frogs were notably elevated (10-502 ng/g; Karasov *et al.* 2005) and an area contaminated by a smoke plume from a PCB warehouse fire (analyzed as composite samples of green frogs (*Rana clamitans*) and leopard frogs, 50-112 ng/g; Phaneuf *et al.* 1995). Sum PCB concentrations at AOC locations were lower than mean concentrations reported in leopard frogs collected from seven lower Great Lakes sites in the mid-1990s (range=2.8-15.7 ng/g, based on 1.2% lipid content and a factor of 0.8 for conversion of 1:1 PCBs to sum PCBs; Gillan *et al*. 1998). Sum PCB concentrations were also lower in St. Clair River AOC frogs compared to frogs from the Detroit River AOC located downstream where mean concentrations at six sites in 2008/09 ranged from 1.5-17.7 ng/g (Hughes *et al.* 2014a). These spatial data in frogs are consistent with relatively lower PCB concentrations found in suspended sediments from the St. Clair River and Lake St. Clair compared to

concentrations in the Detroit River (Jia *et al.* 2010). With respect to concentrations associated with toxic effects in amphibians, no evidence of toxicity was found in northern leopard frogs with a sum PCB body burden of 152 ng/g (Huang *et al.* 1999). Wood frog tadpoles exposed to PCB-contaminated sediment for 42 days had an increased mortality rate of 10%, increased behavioural abnormalities and a body burden of over 22,000 ng/g while those exposed to control sediment had less than 2% mortality and a body burden of 24 ng/g (Savage *et al*. 2002). Given that PCB body burdens in this study were far below these concentrations, it is highly unlikely that current PCB body burdens in frogs from the AOC are associated with toxic effects.

Concentrations of organochlorine pesticides and OCS in St. Clair River AOC frogs were low with mean concentrations of total DDT generally below 4 ng/g and concentrations of other compounds below 1 ng/g. Comparable concentrations of total DDT were found between leopard frogs from the St. Clair River AOC and the Detroit River AOC where means ranged from 0.65-6.45 ng/g in 2008/09 (Hughes *et al.* 2014a). In this study, *p,p'*-DDE concentrations were relatively higher than concentrations in green frogs collected from three sampling locations in British Columbia (range for pooled samples=0.2-0.9 ng/g; Loveridge *et al.* 2007) and yet were several magnitudes lower than concentrations reported in spring peepers (*Pseudacris crucifer*) from Point Pelee National Park where DDT was used 26 years prior in recreational areas for mosquito control (mean=1001 ng/g; Russell *et al.* 1995). DDT concentrations in all St. Clair River frogs were well below the lowest reported toxic effect concentration of 2,400 ng/g in common frog (*R. temporaria*) tadpoles (Cooke 1972). Largely comparable concentrations of DDT and PCBs found in St. Clair River frogs likely reflect the biphasic life history of this species and the primary difference in exposure through food sources (i.e., aquatic versus terrestrial) between the two compounds.

Both HCB and OCS (which is not a pesticide but a by-product of industrial processes) have been identified as contaminants of concern in the St. Clair River AOC (Ontario Ministry of Environment and Energy and Michigan Department of Natural Resources 1995). While detected at low concentrations, HCB was found most consistently in frogs from all study sites while OCS was found in frogs from four of seven AOC locations. Chlordane compounds, dieldrin, and heptachlor are associated with historic agricultural activity and variability in exposure to these compounds was evident in frogs among study sites.

Wildlife consumers may be at an increased health risk due to consumption of frogs at one AOC site, Johnston Channel, where exceedences of the guideline for total DDT (CCME 2001) were noted in two pooled frog samples in 2006. In snapping turtle eggs collected from Walpole Delta in 2007 and 2011, the guideline for total DDT was also exceeded in 18% and 30% of clutches, respectively (Hughes *et al.* 2014b). PCB body burdens in all frog samples however were well below the IJC Aquatic Life Guideline of 100 ng/g for the protection of fish-eating wildlife (IJC 1988). In addition, concentrations of other compounds including sum chlordane, HCB, mirex, dieldrin, heptachlor epoxide and octachlorostyrene at all AOC locations were at most one-fiftieth of fish flesh criteria guidelines for the protection of piscivorous wildlife (Newell *et al.* 1987).

Amphibians act as a conduit for persistent contaminants in the food web as both carnivores and as important food organisms for higher tropic-level species. Concentrations of contaminants such as *p,p'*-DDE and PCBs in leopard frogs in this study were substantially lower than concentrations reported in other local Great Lakes wildlife species which occupy a higher trophic position and have a longer life span. For example, mean sum PCB concentrations in liver of mink (*Mustela vison*) trapped in the AOC and eggs of black terns (*Chlidonias niger*), Forster's terns (*Sterna forsteri*), and herring gulls (*Larus argentatus*) collected from AOC locations from 1991 to 2002 ranged from 82 ng/g-18,200 ng/g (Martin *et al.* 2006; EC unpublished data). Similarly, *p,p'*-DDE concentrations in these four species were also high ranging from 12 ng/g-5,200 ng/g. Mean sum PCBs and *p,p'*-DDE concentrations in clutches of snapping turtle eggs collected from the Walpole Delta in 2007 were at least sixty-five times and four times higher, respectively, than concentrations in frogs from most AOC locations in 2006/07. Johnston Channel was the one exception where similar mean *p,p'*-DDE concentrations were found between the two species (EC unpublished data).

Polybrominated diphenyl ether flame retardants are of emerging concern in the environment and have been associated with disruption of thyroid function and developmental effects in amphibians (McConnell and Sparling 2010). For instance, Carlsson *et al.* (2007) demonstrated that exposure to the congener BDE-99 (one of the predominant congeners in this study) had an impact on metamorphosis in the West African clawed frog (*Xenopus tropicalis*). Given that sum PBDEs were found at relatively lower concentrations compared to sum PCBs and that PBDEs and PCBs are structurally similar and show similar toxic effects, PBDE concentrations in frogs were likely below thresholds associated with toxicity.

Historical releases of mercury from industrial and municipal point sources in the upper reaches of the St. Clair River have contaminated water, sediment and biota downstream along the river and in Lake St. Clair (Ontario Ministry of Environment and Energy and Michigan Department of Natural Resources 1995). Significant differences in mercury concentrations were found in which frogs from two AOC locations, Bassett Channel and Goose Lake, had elevated body burdens compared to those from other study sites including reference sites. In the case of Bassett Channel, increased exposure may have been related to increased St. Clair River water flow carrying more mercury-laden sediment/water from upriver sources compared to the other main Walpole Delta channels where frogs were also caught. Mercury concentrations in sediment collected both in 2005 (GLIER & DBS 2008) as well as in 2011 exceeded the federal sediment quality guideline probable effect level (PEL) at the top and/or mouth of Bassett Channel which coincides well with elevated mercury concentrations in frogs reported in this study. Similarly, a clutch of snapping turtle eggs from the mouth of Goose Lake had a total mercury concentration which was among the highest found in eggs collected from numerous locations in the Walpole Delta in 2011 (Hughes *et al.* 2014b).

Mercury concentrations in frogs from most study sites were on par with concentrations reported in other amphibian species in North America. Similar mercury concentrations were found in one-year-old green frog and bullfrog (*Rana catesbeiana*) tadpoles from Acadia National Park (ANP) in Maine in 2003 (means=25 ng/g and 19 ng/g, respectively; Bank *et al.* 2007). One- to three-year-old larval northern two-lined salamanders (*Eurycea bislineata bislineata*) from ANP had relatively higher concentrations likely as a result of their more invertebrate-based diet (Bank *et al*. 2005; mean=66 ng/g). A slightly higher mean

total mercury concentration of 184 ng/g dry weight (dw) in carcasses (without guts) of southern leopard frog (*Rana sphenocephala*) tadpoles from wetlands in South Carolina was found by Unrine *et al.* (2005) compared to the overall mean mercury concentration of 156 ng/g dw in frogs from AOC locations in this study. In a dosing study in which southern leopard frog tadpoles were exposed to experimental diets of mercury, total mercury body burdens of 200-400 ng/g dw were associated with adverse effects including increased mortality, malformations, and increased time to tail resorption (Unrine *et al.* 2004). Young-of-year northern leopard frogs from Bassett Channel and Goose Lake had mean mercury concentrations within this range (320 ng/g and 226 ng/g dw, respectively) which suggests that they might be exposed to toxicologically significant quantities of mercury at these locations. However, it is difficult to provide an assessment of potential effects associated with mercury exposure in northern leopard frogs in this study since very little research has been done on the effects of mercury in amphibians. In addition, there is evidence that amphibian species may vary in their sensitivity to mercury (Wada *et al.* 2011).

As a component of total mercury, methylmercury is the most biologically relevant form of mercury which is associated with toxicity in biota. Since concentrations of methylmercury were not measured in frogs in this study, estimates of methylmercury concentrations were made in order to assess potential risk to wildlife consumers. These were derived based on mercury data (as wet weights) for composite samples of bullfrog and green frog tadpoles (age, approximately one year old) collected from nine locations in ANP in Maine (Bank *et al.* 2007) and which, in the case of total mercury concentrations, were comparable to body burdens reported in this study. A significant linear relationship was found between concentrations of methylmercury (MeHg) and total mercury (THg) in these nine samples: MeHg=0.222 x THg + 0.439; r=0.75, p=0.020. Using this relationship to estimate methylmercury concentrations in this study, no frogs from the St. Clair River AOC exceeded the methylmercury tissue residue guideline of 33.0 ng/g ww for the protection of wildlife consumers of aquatic biota (CCME 2001). The maximum estimated methylmercury concentration (23.9 ng/g ww) was for a frog from Bassett Channel.

Human populations who frequently consume bullfrogs with elevated concentrations of contaminants may be at an increased health risk (Gerstenberger and Pearson 2002). Since contaminant burdens in leopard frogs may be reflective of those found in bullfrogs, comparisons to human consumption guidelines established for Ontario sport fish were performed. The highest mercury concentration in a leopard frog from this study (106 ng/g wet weight) was well below first consumption guidelines for sensitive (260 ng/g) and general populations (610 ng/g; MOE 2011). Similarly, the maximum sum PCB concentration in a pooled leopard frog sample was approximately one-third that of the first consumption guideline for sensitive populations (105 ng/g; MOE 2011). While frogs collected in this study were young-of-year and approximately five months of age, longer-lived adults could potentially have higher body burdens of contaminants. It is unlikely however that these burdens would approach guideline concentrations associated with sport fish consumption restrictions but this is dependent on the level of contamination in the diet.

Conclusions

Effects on reproduction and development associated with elevated contaminant exposure were largely not evident in an extensive four year study of northern leopard frogs in the St. Clair River AOC (Ontario). Hatching success of embryos was high and frequencies of embryonic deformities were generally low at AOC locations in both exposure studies with statistically similar frequencies reported at non-AOC Great Lakes reference sites. The prevalence of deformities in wild populations of newly-transformed froglets ranged widely based on 18 surveys at seven AOC locations over four study years (0-11.6%). Deformity frequencies at two AOC locations (Chematogan Channel and Bay Lodge) in 2007 were significantly higher compared to the reference sites where they also exceeded the 5% threshold which is considered elevated in wild populations. This finding was not repeated in subsequent surveys of Chematogan Channel and Bay Lodge in 2011 and/or 2014 where the prevalence of deformities was well below this threshold. While considered as high, overall prevalence of testicular oocytes in male young-of-year frogs from AOC locations was lower than frequencies reported in male frogs from other agricultural (non-AOC) sites in southern Ontario. Contaminant body burdens in frogs were generally low for PCBs and other organochlorines and below concentrations associated with toxicity. Mercury body burdens were relatively higher in frogs and most notably at Bassett Channel and Goose Lake. Concentrations of mercury (estimated, as methylmercury), PCBs and most other organochlorines in frogs were below respective tissue residue guidelines for wildlife consumers. Total DDT was the one exception where exceedences of the guideline were noted in two pooled frog samples from Johnston Channel in 2006. Vitellogenin, a protein normally produced by females for egg production, was not detected in male frogs from study sites. Concentrations of atrazine in water were low, varied among study sites, and were not directly associated with the increased prevalence of testicular ooctyes in males from AOC locations. Other agricultural herbicides and nutrients in water were below federal guidelines for the protection of aquatic life while concentrations of some trace metals, such as chromium and cadmium, occasionally exceeded these guidelines but were likely below those considered toxic to amphibians.

In summary, no effects on reproduction and development were found in embryos raised in the laboratory and body burdens of organochlorines in wild populations of frogs were well below those associated with adverse effects on reproduction and development. Deformity frequencies in froglets were low overall based on multiple surveys of many AOC locations over four years and also relative to frequencies reported from surveys conducted at other Great Lakes AOC locations. Mercury concentrations were likely below effect levels however little is known with respect to potential effects associated with mercury exposure in amphibians. Elevated frequencies of males with testicular ooctyes at some AOC locations in this study were within ranges reported outside of the AOC and, as such, cannot be attributed to stressors within the AOC only. Overall the results of this study provide no evidence of impairment of reproductive health and development in wild populations of northern leopard frogs that can be attributed to high level of contaminants in the St. Clair River AOC (Ontario).

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APPENDICES

Appendix 1. Listing of St. Clair River AOC (including WIFN) locations and upstream Lake Huron reference sites, coordinates and corresponding years when surveys, samplings and/or exposure studies were conducted. Surveys of newly-transformed froglets for deformities were conducted in the summer while surveys of pre-hibernation young-of-year frogs for gonad histology, vitellogenin and contaminant body burdens studies were conducted in the fall. Sampling years for analyses of pesticides, nutrients and metals in water are also indicated where the number of analyses conducted in the year is shown in brackets. Note that Heritage and White Bread, located on opposite sides of the Chenal Ecarte, are considered as one location in this report.

Location*	Site	Latitude-Longitude	Embryonic Exposure - Water	Embryonic Exposure - Sediment	YOY Deformities	Gonad Histology	Vitellogenin in Blood	Contaminants Body Burden	Water Chemistry	
									Pesticides & Nutrients**	Metals
WIFN	Bassett Channel	42°32’50.72”N, 82°34’59.59”W	-	-	-	2006, 2007	2006, 2007	2006	2006 (3), 2007 (1)	2006
WFIN	Goose Lake	42°31’00.04”N, 82°30’29.40”W	-	-	2007, 2011. 2014	2006, 2007	2006, 2007	2006	2006 (3), 2007 (1)	2006
WIFN	Heritage	42°38’13.81”N, 82°30’12.85”W	-	-	-	-	-	2006	2006 (2)	2006
WIFN	Johnston Channel	42°32’01.86”N, 82°27’04.58”W	-	-	-	2006, 2007	2006, 2007	2006	2006 (1), 2007 (1)	-
WIFN	Chematogan Channel	42°32’51.08”N, 82°32’40.45”W	-	-	2007, 2011, 2014	2007	2007	2007	2007 (2)	-
WIFN	Dyke Road	42°32’05.12”N, 82°28’26.63”W	-	-	2006, 2007, 2011, 2014	-	-	-	2007 (1)	-
WIFN	Black Shack Rd	42°31’55”N, 82°27’0”W	-	-	2011	-	-	-	-	-
WIFN	St. Anne Island	42°32’6”N, 82°25’31”W	-	-	2011	-	-	-	-	-
WIFN	Top of Chenal Ecarte	42°38’14.94”N, 82°30’10.14”W	-	2011	-	-	-	-	-	-
WIFN	Chenal Ecarte	42°35’8.94”N, 82°26’20.94”W	-	2011	-	-	-	-	-	-
WIFN	Mouth of Chenal Ecarte	42°29’19.50”N, 82°26’7.86”W	-	2011	-	-	-	-	-	-
WIFN	Bassett Channel	42°32’58.32”N, 82°35’2.10”W	-	2011	-	-	-	-	-	-
WIFN	Mouth of Bassett Ch.	42°30’19.62”N, 82°35’2.76”W	-	2011	-	-	-	-	-	-
Non-WIFN	White Bread	42°38’13.81”N, 82°30’12.85”W	2007	-	-	-	-	-	2007 (1)	2007
Non-WIFN	Bay Lodge	42°27’24.92”N, 82°24’45.16”W	2007	-	2006, 2007, 2014	2006, 2007	2006, 2007	2007	2006 (1), 2007 (3)	2007
Non-WIFN	Bear Creek	42°31’58.95”N, 82°23’58.56”W	2007	-	2006, 2007, 2014	2006, 2007	2006, 2007	2007	2006 (1), 2007 (3)	2007
SCR	Sarnia	42°59’55.97”N,	2007	-	-	-	-	-	2007 (1)	2007

Location*	Site	Latitude-Longitude	Embryonic Exposure - Water	Embryonic Exposure - Sediment	YOY Deformities	Gonad Histology	Vitellogenin in Blood	Contaminants Body Burden	Water Chemistry Pesticides & Nutrients**	Metals
		82°25’19.69”W								
SCR	Talfourd Creek	42°54’15.05”N, 82°27’28.97”W	2007	-	-	-	-	-	2007 (1)	2007
SCR	Baby Creek	42°50’03.95”N, 82°28’05.48”W	2007	-	-	-	-	-	2007 (1)	2007
SCR	Terra	42°48’55.83”N, 82°28’33.21”W	-	-	-	-	-	-	2006 (2)	2006
SCR	Clay Creek	42°45’01.42”N, 82°27’59.92”W	2007	-	-	-	-	-	2007 (1)	2007
SCR	Marshy Creek	42°40’49.28”N, 82°29’51.08”W	2007	-	-	-	-	-	2007 (1)	2007
Ref	Port Franks	43°14’03.20”N, 81°53’53.23”W	-	2011	2006, 2007, 2011, 2014	2006, 2007	2006, 2007	2006	2006 (3), 2007 (2)	2006
Ref	Wood Road	43°10’39.24”N, 82°01’09.89”W	2007	-	2006, 2007	2006, 2007	2006, 2007	2006	2006 (3), 2007 (3)	2006, 2007

* Indicates location of site as either on Walpole Island First Nation Territory (WIFN), outside of WIFN but within AOC (non-WIFN), on the St. Clair River (SCR) or as an upstream reference site on Lake Huron (Ref)

** Number in brackets denotes number of sampling periods conducted in a year (i.e., May, June/July and/or September/October).

Appendix 2. Summary of the types of deformities observed in individual northern leopard frog tadpoles exposed to water and sediment from five St. Clair River AOC locations and one upstream reference site on Lake Huron (Port Franks). Each row represents an individual tadpole with at least one deformity of which type(s) observed is denoted by "Y". Tadpoles were staged according to Gosner (1960).

Location	Tadpole Stage	Total Deformities	Gut Formation	Asymmetry	Lateral Tail Flexure	Stunted	Dorsal Tail Flexure	Abdominal Edema	Head Edema	Blisters	Eye Incom-plete	Eye Missing	Skin	Other
Bassett Channel	23	5		Y	Y	Y	Y				Y			
	23	1												Y
	24	1			Y									
	24	1		Y										
	24	1			Y									
	24	1			Y									
	24	4		Y		Y			Y		Y			
	23	4	Y	Y		Y			Y					
	23	4			Y	Y	Y				Y			
	23	3	Y		Y	Y								
	Total	**25**	**2**	**4**	**6**	**5**	**2**	**0**	**2**	**0**	**3**	**0**	**0**	**1**
Chenal Ecarte	24	1			Y									
	24	1			Y									
	23	5	Y	Y	Y	Y					Y			
	24	1				Y								
	23	2	Y			Y								
	24	2		Y	Y									
	23	2			Y	Y								
	Total	**14**	**2**	**2**	**5**	**4**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**
Mouth Bassett Channel	23	4		Y	Y		Y				Y			
	25	4				Y	Y	Y				Y		
	23	3			Y	Y					Y			
	24	1					Y							
	23	1			Y									
	23	7	Y		Y	Y	Y	Y		Y	Y			
	24	2	Y			Y								
	25	5			Y	Y	Y	Y			Y			
	23	6	Y		Y	Y	Y	Y			Y			
	23	7	Y	Y	Y	Y		Y	Y		Y			
	24	1			Y									
	Total	**41**	**4**	**2**	**8**	**7**	**6**	**5**	**1**	**1**	**6**	**1**	**0**	**0**

Location	Tadpole Stage	Total Deformities	Gut Formation	Asymmetry	Lateral Tail Flexure	Stunted	Dorsal Tail Flexure	Abdominal Edema	Head Edema	Blisters	Eye Incom-plete	Eye Missing	Skin	Other
Mouth Chenal Ecarte	24	1										Y		
	24	1			Y									
	23	1			Y									
	23	4	Y	Y				Y	Y					
	24	4	Y	Y				Y	Y					
	23	1							Y					
	24	7		Y	Y	Y	Y		Y		Y		Y	
	Total	**19**	**2**	**3**	**3**	**1**	**1**	**2**	**4**	**0**	**1**	**1**	**1**	**0**
Top Chenal Ecarte	23	1									Y			
	23	3	Y		Y	Y								
	23	5				Y	Y			Y		Y	Y	
	23	5		Y		Y	Y	Y			Y			
	23	1									Y			
	24	2		Y	Y									
	24	3			Y		Y						Y	
	24	1			Y									
	24	1			Y									
	Total	**22**	**1**	**2**	**5**	**3**	**3**	**1**	**0**	**1**	**2**	**1**	**2**	**0**
Port Franks	23	2		Y						Y				
	23	2	Y	Y										
	23	4				Y	Y	Y			Y			
	23	2	Y								Y			
	23	6			Y	Y	Y		Y	Y	Y			
	23	4	Y		Y		Y		Y					
	Total	**20**	**3**	**2**	**2**	**2**	**3**	**1**	**2**	**2**	**3**	**0**	**0**	**0**
Overall Total		**141**	**14**	**17**	**29**	**22**	**15**	**9**	**9**	**4**	**17**	**17**	**3**	**1**

Appendix 3. Concentrations of phenoxy-acid and neutral herbicides in water (ng/L) from St. Clair River AOC (including WIFN) locations and upstream Lake Huron reference sites for three sampling periods (i.e., May, June/July and/or September/October where data are available) in 2006 and 2007. Nutrient (nitrate, ammonia, phosphate) concentrations are also shown (mg/L). ND indicates sample concentration was below the limit of detection; NA indicates that data are not available and NM indicates that water was not collected from the location (i.e., Not Measured). Data for each year are separated by a slash (i.e., 2006/2007) and "-" indicates that data were not collected in either study year. Canadian Water Quality Guidelines (CWQG) for the protection of aquatic life are also provided for each compound (CCME 2007).

		St. Clair River Area of Concern							
		WIFN Location					Other Locations		
Compound	**CWQG**	**Bassett Channel**	**Goose Lake**	**Heritage-White Bread***	**Johnston Channel**	**Chema-togan**	**Dyke**	**Sarnia**	**Talfourd Creek**
Clopyralid	4,000								
May/May		ND/NM	ND/NM	ND/ND	-	-	NM/ND	NM/ND	NM/ND
June/July		ND/NM	ND/NM	ND/NM	-	NM/3.83	-	-	-
Sept./Oct.		1.2/ND	1.64/ND	-	1.11/ND	NM/ND	-	-	-
Dicamba	10,000								
May/May		ND/NM	ND/NM	ND/ND	-		NM/ND	NM/ND	NM/ND
June/July		ND/NM	2.43/NM	0.98/NM	-	NM/ND	-	-	-
Sept./Oct.		ND/ND	ND/ND	-	ND/ND	NM/ND	-	-	-
2,4-D	4,000								
May/May		5.00/NM	24/NM	2.82/3.52	-	-	NM/ND	NM/3.10	NM/8.44
June/July		ND/NM	5.42/NM	3.12/NM	-	NM/27.3	-	-	-
Sept./Oct.		1.69/ND	3.77/ND	-	1.79//ND	NM/ND	-	-	-
2,4-DP	4,000								
May/May		1.42/NM	2.83/NM	ND/ND	-	-	NM/ND	NM/ND	NM/3.85
June/July		ND/NM	1.66/NM	ND/NM	-	NM/ND	-	-	-
Sept./Oct.		ND/ND	ND/ND	-	ND/ND	NM/ND	-	-	-
MCPA	2,600								
May/May		ND/NM	ND/NM	ND/ND	-		NM/ND	NM/ND	NM/ND
June/July		ND/NM	ND/NM	ND/NM	-	NM/ND	-	-	-
Sept./Oct.		ND/ND	ND/ND	-	ND/ND	NM/ND	-	-	-
Bromoxynil	5,000								
May/May		ND/NM	ND/NM	ND/ND	-		NM/ND	NM/ND	NM/ND
June/July		ND/NM	ND/NM	ND/NM	-	NM/ND	-	-	-
Sept./Oct.		ND/ND	ND/ND	-	ND/ND	NM/ND	-	-	-
Mecoprop	4,000								
May/May		ND/NM	ND/NM	ND/ND	-		NM/ND	NM/ND	NM/ND
June/July		ND/NM	ND/NM	ND/NM	-	NM/39.9	-	-	-
Sept./Oct.		ND/ND	ND/ND	-	ND/ND	NM/ND	-	-	-
IPYR	NA								
May/May		ND/NM	ND/NM	ND/ND	-		NM/ND	NM/ND	NM/ND
June/July		ND/NM	ND/NM	ND/NM	-	NM/ND	-	-	-
Sept./Oct.		ND/ND	ND/ND	-	ND/ND	NM/ND	-	-	-
Atrazine	1,800								
May/May		24.2/NM	86.2/NM	27.4/21.0	-	-	NM/21.0	NM/20.4	NM/26.1
June/July		72.8/NM	86.9/NM	-	-	NM/51.6	-	-	-
Sept./Oct.		14.4/14.2	15.4/11.1	-	13.7/16.7	NM/15.8	-	-	-
D-atrazine	1,800								
May/May		ND/NM	37.7/NM	ND/ND	-	-	NM/ND	NM/ND	NM/ND
June/July		51.5/NM	51/NM	-	-	NM/42.9	-	-	-
Sept./Oct.		ND/ND	ND/ND	-	ND/ND	NM/ND	-	-	-
Metolachlor	7,800								
May/May		ND/NM	46.9/NM	ND/ND	-	-	NM/ND	NM/ND	NM/ND
June/July		44.8/NM	70.9/NM	-	-	NM/ND	-	-	-
Sept./Oct.		ND/ND	ND/ND	-	ND/ND	NM/ND	--	-	-
Simazine	10,000								
May/May		ND/NM	ND/NM	ND/ND	-	-	NM/ND	NM/ND	NM/ND

		St. Clair River Area of Concern							
		WIFN Location					Other Locations		
Compound	**CWQG**	**Bassett Channel**	**Goose Lake**	**Heritage-White Bread***	**Johnston Channel**	**Chema-togan**	**Dyke**	**Sarnia**	**Talfourd Creek**
June/July		ND/NM	ND/NM	-	-	NM/ND	-	-	-
Sept./Oct.		ND/ND	ND/ND	-	ND/ND	NM/ND	--	-	-
Metribuzin	1,000								
May/May		ND/NM	ND/NM	ND/ND	-	-	NM/ND	NM/ND	NM/ND
June/July		ND/NM	ND/NM	-	-	NM/ND	-	-	-
Sept./Oct.		ND/ND	ND/ND	-	ND/ND	NM/ND	--	-	-
Nitrates	13								
May/May		NA/NM	NA/NM	NA/0.358	NA/NM	-	NM/0.011	NM/0.41	NM/0.70
June/July		0.26/NM	0.03/NM	0.33/NM	NM/NM	NM/0.009	-	-	-
Sept./Oct.		0.23/NM	0.01/NM	-	0.29/NM	-	-	-	-
Ammonia	1.37								
May/May		NA/NM	NA/NM	NA/0.013	NA/NM	-	NM/0.028	NM/0.008	NM/0.06
June/July		0.08/NM	0.09/NM	0.03/NM	-	NM/0.088	-	-	-
Sept./Oct.		0.06/NM	0.06/NM	-	0.03/NM	-	-	-	-
Phosphate	NA								
May/May		0.011	0.018/NM	0.002/0.004	-	-	NM/0.097	NM/0.004	NM/0.03
June/July		0.010	0.011/MN	0.005/NM	-	NM/0.039	-	-	-
Sept./Oct		0.011/NM	0.010/NM	-	0.006/NM	-	-	-	-

* Heritage is located in WIFN Territory and was sampled in 2006 while White Bread is not in WIFN Territory and sampled in 2007.

Appendix 3 (continued).

		St. Clair River Area of Concern Other Locations (Continued)						Reference Sites	
Compound	**CWQG**	**Baby Creek**	**Clay Creek**	**Marshy Creek**	**Bay Lodge**	**Bear Creek**	**Terra**	**Port Franks**	**Wood Road**
Clopyralid	4,000								
May/May		NM/ND	NM/ND	NM/ND	NM/ND	NM/ND	ND/NM	1.69/NM	ND/ND
June/July		-	-	-	NM/5.73	NM/3.95	ND/NM	10.9/ND	4.16/ND
Sept./Oct.		-	-	-	ND/ND	ND/ND	-	ND/ND	1.21/ND
Dicamba	10,000								
May/May		NM/1.62	NM/1.83	NM/0.90	NM/32.7	NM/8.68	ND/NM	2.25/NM	ND/1.93
June/July		-	-	-	NM/ND	NM/103	ND/NM	744/15.4	11/ND
Sept./Oct.		-	-	-	ND/ND	4.15/ND	-	ND/ND	ND/ND
2,4-D	4,000								
May/May		NM/6.08	NM/5.08	NM/2.74	NM/13.6	NM/157	4.41/NM	13.7/NM	12.2/7.62
June/July		-	-	-	NM/ND	NM/16.3	ND/NM	82.8/ND	17/ND
Sept./Oct.		-	-	-	6.66/ND	95/ND	-	1.44/ND	2.43/ND
2,4-DP	4,000								
May/May		NM/ND	NM/ND	NM/ND	NM/1.16	NM/1.81	1.28/NM	2.12/NM	ND/ND
June/July		-	-	-	NM/4.11	NM/5.35	ND/NM	3.66/ND	2.12/ND
Sept./Oct.		-	-	-	0.7/ND	16.4/ND	NM	1.28/ND	ND/ND
MCPA	2,600								
May/May		NM/ND	NM/ND	NM/ND	NM/ND	NM/3.45	ND/NM	ND/NM	ND/ND
June/July		-	-	-	NM/ND	NM/ND	ND/NM	ND/ND	ND/ND
Sept./Oct.		-	-	-	ND/ND	8.21/ND	-	ND/ND	ND/ND
Bromoxynil	5,000								
May/May		NM/3.63	NM/ND	NM/ND	NM/ND	NM/4.76	ND/NM	ND/NM	ND/ND
June/July		-	-	-	NM/ND	NM/ND	ND/NM	ND/ND	ND/ND
Sept./Oct.		-	-	-	ND/ND	ND/ND	-	ND/ND	ND/ND
Mecoprop	4,000								
May/May		NM/ND	NM/ND	NM/ND	NM/3.58	NM/ND	ND/NM	2.34/NM	ND/ND
June/July		-	-	-	NM/ND	NM/ND	ND/NM	ND/ND	13.4/ND

		St. Clair River Area of Concern Other Locations (Continued)						Reference Sites	
Compound	**CWQG**	**Baby Creek**	**Clay Creek**	**Marshy Creek**	**Bay Lodge**	**Bear Creek**	**Terra**	**Port Franks**	**Wood Road**
Sept./Oct.		-	-	-	ND/ND	1.69/ND	-	ND/ND	ND/ND
IPYR	NA								
May/May		NM/ND	NM/ND	NM/ND	NM/ND	NM/ND	ND/NM	1.63/NM	ND/ND
June/July		-	-	-	NM/ND	NM/ND	ND/NM	11.3/ND	ND/ND
Sept./Oct.		-	-	-	ND/ND	ND/ND	-	ND/ND	ND/ND
Atrazine	1,800								
May/May		NM/213	NM/23.6	NM/31.9	NM/236	NM/41	31.3/NM	184/NM	53/19.8
June/July		-	-	-	NM/103	NM/378	98.5/NM	1610/161	115/16.8
Sept./Oct.		-	-	-	27.8/19.5	48.6/26.8	-	11.5/7.3	13.2/14.2
D-atrazine	1,800								
May/May		NM/ND	NM/ND	NM/ND	NM/36.5	NM/29.3	27.6/NM	51.5/NM	29.2/ND
June/July		-	-	-	NM/53.6	NM/133	57.8/NM	322/42.5	112/ND
Sept./Oct.		-	-	-	ND/ND	48.4/32.9	-	ND/ND	ND/ND
Metolachlor	7,800								
May/May		NM/156	NM/ND	NM/ND	NM/127	NM/173	ND/NM	156/NM	ND/ND
June/July		-	-	-	NM/ND	NM/47.9	128/NM	906/ND	39.5/ND
Sept./Oct.		-	-	-	ND/ND	94.6/ND	NM/NM	ND/ND	ND/ND
Simazine	10.000								
May/May		NM/ND	NM/ND	NM/ND	NM/ND	NM/ND	ND/NM	ND/NM	ND/ND
June/July		-	-	-	NM/ND	NM/ND	ND/NM	28/ND	ND/ND
Sept./Oct.		-	-	-	ND/ND	ND/ND	NM/NM	ND/ND	ND/ND
Metribuzin	1,000								
May/May		NM/ND	NM/ND	NM/ND	NM/ND	NM/ND	ND/NM	ND/NM	ND/ND
June/July		-	-	-	NM/ND	NM/ND	ND/NM	894/ND	ND/ND
Sept./Oct.		-	-	-	ND/ND	ND/ND	NM/NM	ND/ND	ND/ND
Nitrates	13								
May/May		NM/2.09	NM/1.11	NM/0.38	NA/2.68	NA/8.29	NA/NM	NA/NM	NA/0.096
June/July		-	-	-	NM/0.016	NM/0.022	0.47/NM	4.81/0.015	0.53/0.020
Sept./Oct.		-	-	-	0.02/NM	1.92/NM	-	0.02/NM	0.03/NM
Ammonia	1.37								
May/May		NM/0.016	NM/0.04	NM/0.012	NA/0.116	NA/0.034	NA/NM	NA/NM	NA/0.187
June/July		-	-	-	NM/0.028	NM/0.094	0.10/NM	0.12/0.055	0.07/0.028
Sept./Oct.		-	-	-	0.18/NM	0.05/NM	NM/NM	0.04/NM	0.02/NM
Phosphate	NA								
May/May		NM/0.040	NM/0.042	NM/0.006	NM/0.036	NM/0.069	0.032/NM	0.024/NM	0.059/0.054
June/July		-	-	-	NM/0.037	NM/0.031	0.008/NM	0.017/0.034	0.072/0.011
Sept./Oct		-	-	-	0.036/NM	0.042/NM	-	0.015/NM	0.016/NM