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Mercury Exposure in Double-crested Cormorant Chicks from Lake Erie

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Abstract

Mercury (Hg) concentrations in the Great Lakes have declined since the early 1970s, yet legacy contamination from industrial point sources continues to impact some ecosystems. Double-crested Cormorants (*Nannopterum auritum*) accumulate Hg through their piscivorous diet and may be adversely affected by this exposure to Hg. This study compared Hg burdens between colonies in Lake Erie's western and eastern basins that are more, and less Hg contaminated, respectively. Chicks at Middle Sister Island (west) had red blood cell and feather Hg three times higher than at Mohawk Island (east). Maternal transfer could not explain this difference as egg Hg was similar between sites, implicating post-hatch diet as the Hg source. Stable isotope data indicated a higher trophic position in the west, consistent with higher Hg exposure. These results highlight geographically uneven dietary exposure to Hg and pinpoint west basin cormorants as a particularly Hg-impacted population, notably at early life stages.

Keywords

Mercury (Hg), methylmercury (MeHg), Double-crested Cormorant, maternal transfer, trophic position, stable isotopes, Lake Erie

Summary for Lay Audience

Mercury is a naturally occurring element that can become a pollutant in certain circumstances. In water, it can change into methylmercury, a form that builds up in animals and becomes more concentrated at each step of the food chain. My study asked a simple question: why do cormorant chicks in one part of Lake Erie carry more mercury than those in another?

I compared Double-crested Cormorant chicks from two long-standing colonies: Middle Sister Island in the lake's west basin and Mohawk Island in the east. To see when and how chicks were exposed, I measured mercury in three tissues that record different time windows: eggs (pre-hatch, what mothers pass to embryos), red blood cells (post-hatch, recent meals), and feathers (post-hatch, exposure over the time they form).

Egg mercury was similar at both colonies, suggesting embryos began life with a similar amount of mercury in their bodies. After hatching, however, west basin chicks had about three times more mercury in blood and feathers than east basin chicks. I used natural chemical markers in chick tissues that reflect diet and position in the food web. These markers showed that west basin chicks were feeding higher in the food web, meaning the energy reaching them likely passed through more steps in the food chain. Because methylmercury increases at each step, every extra link gives it more chances to build up before it reaches the chicks.

These results point to diet after hatching, rather than maternal transfer, as the main cause of the difference. In other words, mercury risk is not uniform within a single lake: local hotspots of dietary exposure persist even as overall mercury has declined. Because cormorant chicks respond quickly to changes in what and where they feed, they are practical sentinels for finding places and pathways where mercury remains elevated. Targeting those hotspots can guide monitoring, habitat protection, and fish-consumption advice for people.

Co-Authorship Statement

This research was carried out under the supervision of Dr. Keith A. Hobson and Dr. Brian Branfireun at Western University. Both supervisors provided conceptual guidance, methodological advice, and editorial feedback on study design, data interpretation, and presentation throughout the thesis. Dr. Branfireun edited the thesis. In recognition of their supervisory and intellectual contributions, Drs. Hobson and Branfireun will be listed as co-authors on any manuscripts arising from this thesis.

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Introduction

1.1 Mercury Pollution

1.1.1 Mercury as a global contaminant

Mercury (Hg) is an element of global environmental concern, known for its multi-phase transport, and strong tendency to bioaccumulate to toxic levels in aquatic food webs (Selin 2009). Although it is emitted naturally from sources such as volcanic activity and rock weathering into the atmosphere, industrialization has substantially increased mercury emissions, strengthening atmospheric transport and deposition to ecosystems (Selin 2009). Industrial emissions, notably coal combustion, metal smelting, cement production, and artisanal small-scale gold mining, have roughly tripled to quintupled the amount of mercury moving between the land, atmosphere, and oceans relative to pre-industrial levels (Selin 2009). Emitted mainly as elemental mercury vapour (Hg^0), mercury can travel globally through the atmosphere before eventually oxidizing to reactive forms (Hg^{2+}) and being deposited via precipitation to terrestrial and aquatic ecosystems (Selin 2009). This atmospheric transport means mercury pollution is global and diffuse. Even remote regions (Arctic, open oceans) receive mercury deposition from faraway sources.

1.1.2 Mercury in Lake Erie

Emission control policies in North America and Europe since the mid-1980s have significantly curtailed regional mercury outputs, leading to a 20–38% decline in worldwide atmospheric mercury concentrations over recent decades (Slemr et al. 2011). However, because mercury pollution is a transboundary issue, continued emissions in other regions (and re-mobilization of legacy deposits) sustain an ongoing global cycle of mercury contamination (Selin 2009; Qiu et al. 2025). The Laurentian Great Lakes region demonstrates this dynamic: despite local emission reductions, biologically relevant mercury levels persist due to both legacy industrial sources and long-range atmospheric deposition (Cohen et al. 2016; Janssen et al. 2021).

Mercury levels in Great Lakes fisheries generally do not present a major issue due to having lower concentrations than other freshwater systems (Zhou et al. 2017; Ni et al. 2021). The Great Lakes have experienced mercury contamination in the past, and although levels in biota declined after the 1970s due to pollution controls, progress has stalled or even reversed in recent decades (Zhou et al. 2017). Long-term monitoring in Lake Erie top predator fish species showed that mercury concentrations decreased through the 1970s–2000s but began to level off or increase by 2010 (Bhavsar et al. 2010; Zhou et al. 2017). One hypothesis to explain this trend is an ecological shift caused by invasive species. The late 1980s invasion of dreissenid mussels, including the zebra mussel (*Dreissena polymorpha*) and quagga mussel (*Dreissena bugensis*), may have altered food-web pathways for contaminants. Mussels filter large volumes of water and accumulate mercury in their tissues; round gobies (*Neogobius melanostomus*) then prey on these mussels, effectively shunting mercury from the benthic compartment into the fish community (Hogan et al. 2007; Azim et al. 2011). Thus, changes in ecosystem trophic transfer can affect mercury bioavailability and biomagnification.

Lake Erie is the smallest of the Great Lakes by volume and can be divided into three basins based on limnology and bathymetry (Reutter 2019). The west basin is shallow (mean depth 7.4 m, maximum depth 19 m), eutrophic, and receives water inflows dominantly from the Detroit River (Mortimer 1987). It is separated from the central basin by the island chain extending from Point Pelee, Ontario, to Marblehead, Ohio (Bedford and Abdelrhman 1987). The central basin is deeper (mean depth 18.3 m, maximum depth 25 m), mesotrophic, and has recurring hypoxia events, whereas the east basin is the deepest (mean depth 24.4, maximum depth 60 m) and most oligotrophic region of the lake (Mortimer 1987; Karatayev et al. 2022). The central and eastern basins are separated by the Pennsylvania Ridge, which extends southward from the base of Long Point (Ontario) to Erie, Pennsylvania (Boyce et al. 1980).

Regional variation in chemical and physical characteristics across Lake Erie affects ecological processes and contaminant distribution. Lake Erie's western basin, in particular, has a legacy of industrial and urban activity near the Detroit–Windsor corridor: sediments and the water column in this region exhibit higher mercury

concentrations than the deeper, less impacted east basin (Dove et al. 2012; Lepak et al. 2015). Historically, a dominant point-source of mercury to the St. Clair–Detroit River corridor (feeding the west basin) was Dow Chemical’s Sarnia chlor-alkali plant on the St. Clair River, which discharged large daily masses of Hg in the mid-20th century. Approximately 13.6 kg of mercury was deposited into the river daily for 20 years (Forsythe et al. 2016).

1.2 Methylmercury in organisms

1.2.1 Exposure and uptake

Once deposited in ecosystems, inorganic mercury enters a complex biogeochemical cycle. In aquatic environments such as lakes, wetlands, and ocean margins, certain microbes (particularly anaerobic sulfate-reducing and iron-reducing bacteria in low-oxygen sediments) can convert inorganic mercury into methylmercury (MeHg; Compeau and Bartha 1985; Fleming et al. 2006). This microbial methylation process is a critical point in mercury’s environmental fate, as MeHg is a highly toxic form that readily bioaccumulates in organisms (Morel et al. 1998). Once formed, MeHg binds strongly to sulfur-containing proteins and efficiently assimilates into aquatic food webs (Morel et al. 1998). Whereas inorganic mercury often becomes trapped in sediments, MeHg enters aquatic food webs and increases in concentration as it moves up the trophic hierarchy. As an example, plankton take up MeHg from water and sediments, small forage fish eat the plankton, predatory fish eat the forage fish, and so on, with each step concentrating the mercury further. Top predators in aquatic systems can therefore have MeHg concentrations millions of times higher than the water they inhabit (Driscoll et al. 2007). This is of particular concern because in vertebrates, even low, sublethal concentrations can impair neurodevelopment, locomotion, immune function, and reproduction (Grandjean et al. 1997; Lewis et al. 2012; Varian-Ramos et al. 2014; Zhu et al. 2020).

Methylmercury is the predominant form of mercury found in avian tissues, comprising approximately 96% of total mercury (THg) in eggs (Ackerman et al. 2013), 92% in feathers (Renedo et al. 2017) and over 80% in whole blood (Rimmer et al. 2005; Álvarez et al. 2013). Birds primarily acquire mercury through their diet, as they consume fish,

invertebrates, or other prey that have bioaccumulated MeHg. Upon ingestion, MeHg is absorbed in the gastrointestinal tract with high efficiency and enters the bloodstream, where it binds mostly to red blood cells (90%) and the rest binds to plasma (Wolfe et al. 1998). Studies on common loon (*Gavia immer*) chicks have shown very high oral bioavailability of ingested MeHg (83%) to systemic circulation (Fournier et al. 2002).

In blood, methylmercury declines biphasically: an initial rapid distribution phase lasting days is followed by a slower terminal phase lasting weeks, with rates strongly modulated by molt because growing feathers sequester MeHg from circulation (Monteiro and Furness 2001). Remaining MeHg in circulation is distributed to organs such as the liver, kidneys, muscles, and brain (Finley et al. 1979). With ongoing dietary exposure, tissues may accumulate MeHg over time as intake exceeds elimination (Monteiro and Furness 2001; Bennett et al. 2009). The physiological mechanisms underlying its clearance are central to understanding MeHg dynamics in avian tissues.

1.2.2 Elimination pathways of methylmercury in birds

Although methylmercury tends to accumulate in birds, there are a few critical pathways by which birds can reduce their internal mercury burden. Two significant elimination routes for MeHg in birds are feather molt and egg laying. During feather growth, circulating mercury is deposited into the keratin structure of feathers (Appelquist et al. 1984). Once the feather is fully grown, it is metabolically inert, effectively locking away the mercury it contains. When the bird subsequently molts and sheds these feathers, a portion of the body's mercury burden is removed. Feather molt can thus be viewed as an excretory process. For example, 93% of adult Bonaparte's Gull (*Chroicocephalus philadelphia*) mercury burdens were found to be in their feathers after the completion of a molt (Braune and Gaskin 1987). This mechanism helps protect vital organs by shunting mercury into a tissue that will be discarded. It is important to note that although feather growth during molt can eliminate mercury, molt is not continuous. Many species replace their feathers once per year, so mercury accumulated between molts largely remains in the body until the next molt-driven period of feather growth.

A second major elimination route is maternal transfer of mercury to eggs (Bond and Diamond 2009). Laying females can transfer part of their body burden of MeHg into the developing eggs, which serves both as a source of exposure for the embryo and a depuration pathway for the mother. A considerable proportion of a female bird's mercury burden can be deposited into its clutch, particularly in species that lay multiple or relatively large eggs (Bond and Diamond 2009). This elimination route is typically not as significant as feathers, with a relatively smaller portion of the maternal burden (13–24% of total body mercury per clutch for some species) being transferred (Lewis et al. 1993; Robinson et al. 2011). Although maternal transfer provides an opportunity for females to reduce their mercury load, it constitutes a relatively minor depuration pathway compared to molt and carries the ecological cost of exposing developing embryos to MeHg.

1.2.3 Maternal transfer to eggs

When female birds deposit mercury into their eggs, it is a double-edged sword: it relieves the mother of some mercury but passes that burden to the developing embryo. Maternal transfer is the exposure route of mercury to offspring pre-hatch; embryos receive mercury directly from the yolk and albumen provided by the mother, so chicks hatch with a mercury burden even before they have had any direct dietary exposure (Ackerman et al. 2020). The amount of mercury transferred to eggs can be substantial. Research synthesizing data across dozens of bird species has confirmed that egg mercury concentrations generally correlate with the mother's own mercury levels (Ackerman et al. 2020). Females with higher blood or tissue mercury tend to produce eggs with higher mercury. However, the efficiency of this transfer is not fixed. It varies by species and depends on the female's mercury load (Ackerman et al. 2020).

One comprehensive meta-analysis examined paired blood and egg samples from 26 bird species in 6 taxonomic orders (Ackerman et al. 2020). The findings showed that as a female's blood mercury increases, the mercury in her eggs also increases, but not in a linear fashion. At higher maternal mercury concentrations, a smaller fraction of the total is allocated to the eggs (Ackerman et al. 2020). This suggests a saturating relationship or a protective mechanism that limits how much mercury a heavily contaminated female can transfer into its eggs. Additionally, clear differences emerged among bird groups. For

example, waterfowl (order Anseriformes) transfer a significantly greater proportion of their mercury to eggs compared to songbirds (order Passeriformes; Ackerman et al. 2020). The analysis quantified that waterfowl females can offload up to 3–4 times more mercury into eggs, relative to their blood level, than do small passerines (Ackerman et al. 2020).

The consequence of maternal transfer is that embryos can experience mercury exposure from the moment of early development. Mercury in the egg can reduce hatchability and cause malformations in embryos (Hoffman and Moore 1979; Burger and Gochfeld 1997). Sensitivity varies by species, but bird embryos are generally more sensitive to the effects of mercury exposure than adults (Barr 1986; Wolfe et al. 1998; Heinz et al. 2009). Mercury's teratogenic effects can kill embryos or cause sublethal problems that carry into post-hatch life, even affecting reproduction as an adult (Paris et al. 2018). The maternal-offspring transfer efficiency directly influences this risk. Species that naturally deposit more mercury into each egg (such as waterfowl) may expose their embryos to higher levels, though paradoxically this strategy may help the mother survive to breed again. In contrast, species that deposit less might spare their eggs at the cost of retaining more mercury themselves.

Maternal transfer establishes the starting point: embryos inherit mercury deposited in yolk and albumen and hatch with mercury already present in circulation. Once feeding commences, however, blood becomes the most responsive indicator of exposure, integrating dietary inputs over weeks while turning over far more rapidly than other tissues (Evers et al. 2005). The following section focuses on blood as a practical and sensitive biomarker of recent mercury intake.

1.2.4 Blood as an indicator of recent exposure

Monitoring mercury in wild birds often relies on sampling tissues that can be collected without harming the bird. Blood is one such tissue, and it is widely used as a biomarker of recent mercury exposure. The rationale for using blood is that it reflects dietary intake over the past several weeks to a few months, providing a measure of the bird's current exposure (Evers et al. 2005). The half-life of MeHg in adult bird blood is on the order of

20–30 days (though it can range roughly 2–8 weeks depending on species and metabolic rate). This means that a bird's blood Hg concentration is influenced by what it has been eating in the last one to two months and will respond to changes in diet or exposure relatively quickly (compared to, say, feathers or claws which reflect older exposure). Because MeHg elimination half-life increases with body mass (JECFA 2000), larger birds are also expected to retain MeHg in their blood for longer than smaller species given similar exposure histories. In chicks, the situation is slightly different: hatchlings have some Hg in their blood derived from the egg (maternal transfer), but as they begin feeding, their own dietary intake starts to dictate blood levels as the effects of maternal transfer are mitigated by body and feather growth (Ackerman et al. 2011).

Another advantage of blood sampling is that it is minimally invasive; a small volume of blood can be taken from a bird (e.g., from the brachial vein) without lasting harm, and the bird can be released. This has made blood mercury a common metric in field studies and long-term monitoring programs. It is important to recognize that blood mercury concentrations can exhibit daily or seasonal variations. In breeding birds, for example, females often have lower blood mercury during egg-laying because they have just transferred some to eggs. Feather growth is another elimination pathway, so blood mercury will be lower after a molting period (Condon and Cristol 2009). Rapid growth in chicks can also dilute mercury as new biomass is added faster than mercury accumulates, temporarily lowering concentrations (Ackerman et al. 2011). Still, blood provides an accurate snapshot of biologically active mercury in the bird, as it is often strongly correlated with internal organ concentrations (Eagles-Smith et al. 2008). Blood thus indexes recent dietary exposure and responds on a timescale of days to weeks. To complement this short-term indicator, feathers provide a time-integrated record of the mercury present in blood during feather growth (Monteiro and Furness 2001).

1.2.5 Feathers as a time-integrated exposure indicator

Feathers are another commonly used tissue for mercury monitoring in birds. In contrast to blood, which reflects recent exposure, fully grown feathers represent a time-integrated record of mercury exposure during the period of feather development. Growing feathers sequester mercury from the bloodstream; once growth is complete and the feather's blood

supply is cut off, the mercury in that feather remains locked in keratin (Appelquist et al. 1984). Feather mercury concentrations can therefore be interpreted as indicators of the bird's blood mercury at the time the feather was formed (Monteiro and Furness 2001).

It is important to understand when and where a feather was formed in order to accurately interpret its mercury concentration. In nestlings, feathers record the mercury present in circulating blood during growth; down in young chicks tracks maternal mercury, whereas later-growing feathers (such as from the tail or wing) mainly reflect mercury from parental provisioning and can thus be used to infer diet-derived exposure during the nestling period and compare site-level contamination (Ackerman and Eagles-Smith 2009; Ackerman et al. 2011; Keller et al. 2014). Feathers are also convenient for field sampling; they can be plucked or collected non-destructively (birds begin to regrow plucked feathers within a few weeks of sampling; Delnatte et al. 2014) and stored easily, making them valuable for large-scale monitoring programs.

1.3 Stable isotopes as ecological tracers

The application of stable isotope analysis in ecology has enabled researchers to examine the trophic interactions and dietary niche of numerous bird species (Inger and Bearhop 2008; Hobson 2009). Stable isotopes are non-radioactive forms of elements that have the same number of protons but a different number of neutrons. As a result, they have the same atomic number but a different atomic weight. Compounds with different isotopic compositions react at different rates. The relative abundance of heavier to lighter stable isotopes of carbon ($^{13}\text{C}/^{12}\text{C}$, expressed as $\delta^{13}\text{C}$ relative to an international standard) and nitrogen ($^{15}\text{N}/^{14}\text{N}$, expressed as $\delta^{15}\text{N}$ relative to an international standard) are commonly used to study trophic relationships of birds and other animals, as the isotopic composition of these elements changes as energy and nutrients are transferred through the food chain (Kelly 2000).

The isotopic ratios in a consumer's tissues reflect those of its food sources, with predictable fractionation occurring at each trophic exchange. Values of $\delta^{13}\text{C}$ are typically higher in benthic versus pelagic primary producers (Hobson et al. 1995; Zimmer et al. 2020) with minimal to no enrichment (0–1‰) with trophic level (Hecky and Hesslein

1995; Vander Zanden and Rasmussen 1999). As such, $\delta^{13}\text{C}$ measurements in consumers can be used to identify the primary sources of energy in aquatic food webs. In contrast, values of $\delta^{15}\text{N}$ generally increase by 3–4‰ with each trophic transfer and can be used to measure an organism's trophic position once baseline $\delta^{15}\text{N}$ values are accounted for and an appropriate trophic enrichment factor is chosen (Post 2002; Perkins et al. 2014, Stephens et al. 2023).

When using stable isotopes to study diet, the choice of tissue is critical because different tissues integrate dietary information over different time scales. Metabolically active tissues have faster isotope turnover, meaning new isotopes are quickly incorporated into the tissues and reflect more recent feeding. For instance, turnover of carbon in bird plasma is very rapid (on the order of days), while carbon in red blood cells and muscle turns over more slowly (over several weeks; Hobson and Clark 1992; Hobson and Clark 1993). Being inert, feathers represent a special case: a feather's isotopic composition is fixed during the short period of its growth and then remains unchanged once the feather is fully formed (Thompson et al. 1998). As a result, a bird's feather preserves the dietary isotope signature from the time and location where that feather grew. Because of these differences, the choice of tissue for isotope analysis must align with the ecological question.

1.4 The Double-crested Cormorant

Double-crested Cormorants (*Nannopterum auritum*) are abundant colonial waterbirds in the Great Lakes. This species has shown remarkable resilience and adaptability in its diet. Studies analyzing the diets of cormorants typically suggest that these birds are opportunistic piscivores, do not exhibit preference towards any specific species, and seldom take commercially valuable fish (Knopf and Kennedy 1981; Hobson et al. 1989; Ludwig et al. 1989; Madenjian and Gabrey 1995; Johnson et al. 2015). Cormorants experienced a remarkable population increase in the Great Lakes region since the continental ban of DDT in the 1970s (Korfanty et al. 1997). This population resurgence has led to persecution and a province-wide cull in Ontario over concerns for game fish populations (Hobson 2021). However, the scientific basis for such management actions is often lacking, particularly considering the complex environmental changes that have

taken place within the Great Lakes ecosystem over recent decades, including repeated non-native species invasions that have restructured food webs and altered nutrient cycling (Diana et al. 2006; Hobson 2021; Ludwig et al. 2023).

1.4.1 Life history and ecology

The Double-crested Cormorant is a colonial waterbird native to North America, widespread across marine coasts and interior freshwaters, with high densities in the Great Lakes (Wires 2014). Adults are dark, medium-large piscivores with an orange–yellow gular region and a hooked bill, foraging as pursuit divers in both nearshore and offshore habitats.

Double-crested Cormorants breeding on the Great Lakes are migratory, wintering predominantly along the Atlantic and Gulf coasts of the United States and returning each spring to breed. Breeding occurs in dense colonies often on predator-limited areas such as small islands. Colony size varies widely, from a few dozen to thousands of pairs, reflecting factors such as habitat quality, prey supply, and predator presence (Wires and Cuthbert 2010). Most individuals first breed at about three years of age with clutches typically consisting of 3 to 5 eggs (Brechtel 1983; Weseloh and Ewins 1994). Males and females share incubation and chick-rearing duties, coordinating frequent feedings that support rapid nestling growth (Brechtel 1983). High breeding site fidelity is also characteristic of cormorants; adults frequently return to the same breeding site each year and are even known to return to areas that are near the colony they were originally hatched at (Dolbeer 1991; Clark et al. 2006).

Population trajectories for Double-crested Cormorants over the twentieth century were strongly shaped by human activities. Colonies declined sharply in mid-century due to organochlorine pollutants, direct persecution, and habitat loss. Following environmental regulation and protective measures, numbers rebounded. In the Great Lakes, populations recovered from near-extirpation in the early 1970s to more than 115,000 breeding pairs by 2000, marking one of the region’s most prominent avian recoveries (Weseloh et al. 2002). This resurgence generated tensions with fisheries and aquaculture interests where

foraging overlaps with species valued by anglers; nonetheless, ecologically it represents the re-establishment of a native upper-trophic predator in restored food webs.

Double-crested Cormorants are opportunistic piscivores, and their diet in the Great Lakes is dictated largely by the local fish community and seasonal availability of prey (Bur et al. 1999; Johnson et al. 2010; Wires 2014). They are pursuit-divers, catching fish by diving from the surface and swimming underwater. Despite being capable of diving up to 25 meters, studies of cormorant foraging have shown that they often feed within a few kilometers of their breeding colony and concentrate on shallow, nearshore areas with abundant small to medium-sized fish (Bur et al. 1999; Stapanian et al. 2002; Coleman et al. 2005; Coleman 2008). This foraging ecology makes cormorants sensitive integrators of local methylmercury in food webs, with burdens varying among colonies.

1.4.2 Mercury exposure in cormorants

Although Double-crested Cormorants have not been as extensively studied for mercury contamination as some other piscivorous birds (Ackerman et al. 2016), several studies provide insight into their mercury exposure and potential risks. Generally, cormorants in the Great Lakes carry moderate levels of mercury that reflect their position as fish predators, but that mercury burden can vary spatially (Lavoie and Campbell 2018).

A large-scale study by Lavoie and Campbell (2018) surveyed cormorant colonies across Canada, including the Great Lakes, and demonstrated that mercury in chick feathers is a good proxy for adult mercury exposure at the same colony. They also observed that colonies differed widely in chick feather mercury, with higher concentrations found in Lake Huron. These results support using nestling feathers as colony-level indicators of mercury exposure and highlight pronounced spatial differences within the Great Lakes.

Research by Gibson et al. (2014) reported that female Double-crested Cormorants in Lake Ontario had blood mercury concentrations sufficient to induce physiological changes; they found a positive correlation between blood THg levels and the expression of oxidative stress-related genes in the birds' blood, suggesting that even sublethal mercury exposure was affecting cormorant cellular processes. This underscores that

mercury burdens in wild cormorants can reach biologically impactful levels, even at concentrations considered low risk.

While cormorant populations have proven resilient and robust, they are unavoidably accumulating mercury as top predators in an aquatic system, and significant spatial or temporal changes in that accumulation warrant careful examination. Understanding mercury exposure in cormorants is not only relevant for their conservation and management, but it also provides insight into the broader mercury dynamics of the Lake Erie ecosystem that could affect other wildlife and human fish consumers.

1.5 Study objectives

Research in Lake Erie has found that cormorant chicks from west basin colonies have higher mercury levels than those from the eastern basin (M. C. R. Robinson, undergraduate thesis), possibly mirroring the west-east gradient in mercury levels observed in some fish species as well as known mercury concentration gradients in sediments and the water column (Azim et al. 2011; Dove et al. 2012; Lepak et al. 2015). Differences in mercury burdens of cormorant chicks can arise through two main exposure pathways: (1) pre-hatch maternal transfer of Hg into yolk and albumen, which can cause chicks to hatch with Hg already present in circulation, and (2) post-hatch dietary uptake of Hg through parental provisioning once feeding begins (Ackerman et al. 2020). Figure 1 summarizes the conceptual logic that links these pathways to the thesis objectives.

When maternal transfer differs between colonies (Figure 1A), eggs (and thus hatchlings) should begin with different Hg burdens (different intercepts), but two post-hatch outcomes are possible. In a maternal transfer only scenario, post-hatch dietary Hg uptake rates are comparable between colonies, so chicks remain separated by a similar offset through time. In a compounded exposure scenario, west-basin chicks not only start higher due to maternal transfer but also experience higher post-hatch dietary Hg intake than east basin chicks, causing trajectories to further diverge after hatching. The “slope uncertainty” in Figure 1A is intended to reflect this uncertainty in post-hatch dietary accumulation rate. In contrast, when maternal transfer is similar between colonies (Figure 1B), eggs (and hatchlings) should start with similar Hg burdens (similar intercepts), and

any subsequent divergence in chick blood and feather Hg would be attributed primarily to post-hatch diet, from differences in ingested prey Hg.

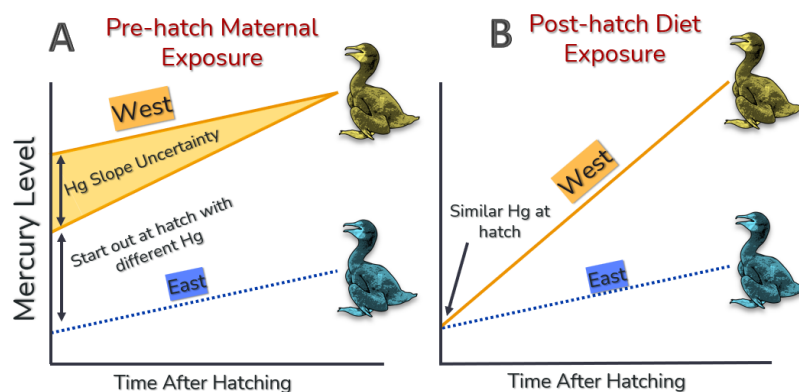


Figure 1. Conceptual model for distinguishing pre-hatch maternal versus post-hatch dietary mercury exposure pathways in Lake Erie Double-crested Cormorant chicks. (A) Pre-hatch maternal exposure differs: west (orange) and east (blue) chicks hatch with different Hg burdens. (B) Post-hatch dietary exposure differs while maternal transfer is similar: chicks hatch with comparable Hg burdens but diverge during chick rearing if dietary exposure differs between colonies.

Using the pre-hatch vs. post-hatch exposure framework in Figure 1 as a guide, I pursued the following objectives:

Objective 1. Quantify and compare mercury concentrations (total Hg and methylmercury) in multiple tissues of Double-crested Cormorants across colonies, including egg components, chick blood, and chick feathers, to characterize mercury exposure at each site and examine how chick mercury exposure differs between pre-hatch (maternal transfer to egg) and post-hatch (dietary ingestion) pathways.

Objective 2. Assess dietary factors (trophic position and benthic feeding reliance) using stable isotopes ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) in cormorant tissues and local prey fish, in order to interpret the observed mercury patterns in bird tissues. The study aims to discern whether higher mercury in a given colony is attributable to higher food-chain position (trophic amplification) and/or reliance on benthic versus pelagic energy pathways that might carry different mercury loads.

2 Methods

2.1 Study sites

Middle Sister Island and Mohawk Island, located in Lake Erie's western and eastern basins, respectively, served as field sites for this study. Middle Sister Island (west basin) is a small (3.8 ha) limestone island in the Pelee Archipelago (Ontario) situated about 21 km southeast of the Detroit River mouth (Figure 2; 41.8487° N, 83.0009° W). It is largely forested with remnant Carolinian vegetation, although heavy guano deposition from nesting waterbirds appears to impair some tree growth. There is no human presence, and the island hosts several colonial waterbird species including American White Pelicans (*Pelecanus erythrorhynchos*), American Herring Gulls (*Larus argentatus*), Great Blue Herons (*Ardea herodias*), and Great Egrets (*Ardea alba*). Mohawk Island (east basin) is a low-lying small (4 ha) limestone shelf in northeastern Lake Erie (off Haldimand County, Ontario) with very sparse vegetation (Figure 2; 42.834° N, 79.523° W). Other colonial waterbirds nesting here include American Herring Gulls, Ring-billed Gulls (*Larus delawarensis*), and Caspian Terns (*Hydroprogne caspia*). Mohawk Island was designated a National Wildlife Area in 1978 and hosts ruins of a 19th-century lighthouse, but there is no human presence currently.

Double-crested Cormorants breed in large numbers on both islands. Middle Sister Island's colony was first established in 1999 and has since become a prominent breeding site in western Lake Erie (Hebert et al. 2005). Cormorant numbers on Middle Sister Island rose from 15 nests in 2000 to an estimated 2,453 nests in 2014 (COSEWIC 2014). Mohawk Island similarly supports one of the largest cormorant colonies in the eastern basin. First colonized in 1983, there were 900 active nests in 2008 (Clark et al. 1983; Environment and Climate Change Canada 2016). Estimates of nesting pairs were 1,200 for Middle Sister Island and 1,750 for Mohawk Island in 2022 (J. Ludwig, Personal Communication, September 7, 2022). The colonies at these two sites provided the basis for comparing west versus east basin conditions.



Figure 2. Study area map of Lake Erie showing the location of Middle Sister Island (west basin) and Mohawk Island (east basin). Basemap: ESRI Ocean; projected in WGS84.

2.2 Sample collection and field procedures

Field sampling took place during the cormorant breeding season of 2024, with archived samples and data incorporated from previous 2022–2023 fieldwork. I collected eggs, chick blood and feathers, as well as regurgitated prey at each site following standardized protocols approved by Western’s Animal Care Committee (Permit #2020-166), Environment and Climate Change Canada (Permit #NF-OR-2024-MINWA-04), and the Ontario Ministry of Natural Resources and Forestry (Permit #1105309).

2.2.1 Egg sampling

Eggs were collected early in the incubation period to investigate maternal mercury transfer. I specifically targeted first-laid eggs in each nest during visits in early May 2024. Double-crested Cormorants incubate their eggs under their feet, causing eggshells to become progressively dirtier over time. A relatively clean single egg in a nest was

therefore presumed to be the first egg of the clutch. To avoid mistaken identification, the area around each nest was checked for any displaced eggs (eggs that might have rolled out), but it is possible that eggs could have been missing due to predation. Presumed first-laid eggs (one per nest) were collected from multiple nests at Middle Sister Island (west basin) and Mohawk Island (east basin). Samples were stored in a cooler for transport, and they were frozen at -20°C until they could be processed. In the laboratory, eggs were opened and the contents divided into albumen and yolk fractions. These fractions were freeze-dried separately and then homogenized to a fine powder using a mortar and pestle.

2.2.2 Chick blood and feather sampling

Chick sampling was conducted in mid-June, when nestlings were approximately 3–5 weeks old (based on size of chick; Dunn 1975). Archived samples from each island were collected one week apart, with a visit to the east basin occurring first. Samples from 2024 were collected 1 day apart, first visiting the west basin. I collected a blood sample by puncturing the brachial wing vein of each chick using a 21-gauge needle and drawing blood into heparinized capillary tubes. The distal ends of a primary wing feather (P10) and central tail feather (R1) were subsequently collected using scissors. Samples were stored in a cooler for transport. Blood samples were processed to separate red blood cells from plasma by centrifuging for 5 minutes at 3000 RPM, since mercury in avian blood is known to be predominantly associated with the cellular portion (Wolfe et al. 1998). Red blood cell samples were frozen (-20°C) and later freeze-dried in the lab prior to chemical analysis.

2.2.3 Regurgitate sampling

Cormorant adults and chicks often regurgitated stomach contents when disturbed. Samples of the regurgitated fish in good condition were collected opportunistically from the ground and stored in a cooler for transport. The fish were measured for fork length and identified to species (Scott and Crossman 1973). A dorsal muscle sample was taken from each fish. These samples were frozen at -20°C and later freeze-dried before stable isotope analysis.

2.3 Laboratory Analysis

2.3.1 Mercury analysis

All mercury analyses were carried out at the Biotron, University of Western Ontario (CALA-accredited, maintaining compliance with ISO/IEC 17025:2017) using methods modified from U.S. EPA protocols. Red blood cell, albumen and yolk samples were freeze-dried. Feathers were washed sequentially with de-ionized water and three times with acetone to remove external contaminants.

2.3.2 Total mercury quantification

Each sample was loaded into a nickel boat and introduced into a Milestone DMA-80 EVO Direct Mercury Analyzer under Thermal Decomposition Atomic Absorption conditions (modified from EPA 7473). All calibration curves exhibited $R^2 > 0.9950$. For red blood cells, albumen, and yolk, instrument performance reference (IPR) checks at low and high levels yielded recoveries of 108% and 98%, respectively, and sample-duplicate relative percent differences averaged 2%. For fish muscle, IPR low was 90% and IPR high was 98%. For feathers, IPR low was 99% and IPR high was 101%. Accuracy was assessed by certified reference material (CRM): ML3 human blood (111.0% recovery for yolk, albumen, and red blood cells), DORM-5 fish protein (89% recovery for fish muscle and 110.8% recovery for yolk, albumen, and red blood cells), IAEA-086 human hair (96% for feathers). Matrix spikes (MS) and matrix spike duplicates (MSD) had recoveries of 104% and 103% with a relative percentage difference (RPD) of 1% for yolk, albumen and red blood cells. Matrix spikes and MSDs for fish were 95% and 95% with an RPD of 0.5%. For feathers, MS and MSD were 103% and 103% with an RPD of 1%. The absolute method detection limit (MDL) and method reporting limit (MRL) were 0.0696 ng and 0.2087 ng. All reported results were above the MRL. THg concentrations were calculated as the measured Hg mass (ng) divided by the sample mass (g) and are reported as ng/g on a dry-weight basis.

2.3.3 Methylmercury quantification

Samples underwent Cold Vapour Atomic Fluorescence Spectroscopy on a Tekran Model 2700 (modified from EPA 1630). Red blood cell, albumen, and yolk samples were first digested in 2.0 mL of 4.0 M HNO₃ and brought to a total volume of 10 mL before further dilution. Fish muscle tissue and feathers were digested with 2 mL of 25% KOH in MeOH solution and brought to a final volume of 10 mL before further dilution. Yolk samples were diluted 200 times (0.15 mL to 30 mL), while red blood cell, albumen, fish muscle, and feather samples were diluted 1,000 times (0.03 mL to 30 mL). Two fish muscle samples initially fell below MRL but were reported above the MRL after being rerun at a 300-fold dilution. All calibration curves exhibited $R^2 > 0.9950$.

For red blood cell, albumen, and yolk samples, IPR (initial precision and recovery) and OPR (on-going precision and recovery) standards averaged recoveries of 95% and 94% (IPR SD = 3%). DORM-4 fish protein CRM recovery averaged 95%. Matrix spikes and MSDs yielded recoveries of 107% and 108% with RPDs of 6% for both sample duplicates and MS/MSD pairs. The MDL was 0.0176 ng and the MRL was 0.0528 ng (based on a 200-fold dilution).

For fish muscle samples, IPR and OPR standards averaged recoveries of 93% and 93% (IPR SD = 2%). DORM-4 fish protein CRM recovery averaged 81%. Matrix spikes and MSDs yielded recoveries of 95% and 100% with RPDs of 20% for sample duplicates and 11% for MS/MSD pairs. The MDL was 0.0229 ng and the MRL was 0.0686 ng (based on a 300-fold dilution).

For feather samples, IPR and OPR standards averaged 91% and 93% (IPR SD = 2%). IAEA-086 human hair recovery averaged 102%. Matrix spikes and MSDs yielded recoveries of 100% and 103% with RPDs of 8% for sample duplicates and 8% for MS/MSD pairs. The MDL was 0.0229 ng and the MRL was 0.0686 ng (based on a 200-fold dilution).

All method blanks measured below the MDL (indicating that instrument and reagent blanks contributed negligible mercury contamination) and all reported results exceeded

the MRL. Methylmercury precision criteria required $RPD < 35\%$ when MeHg concentrations exceeded THg concentrations. Final MeHg concentrations were calculated as the measured MeHg mass (ng) divided by the sample mass (g) and are reported as ng/g on a dry-weight basis.

2.3.4 Stable isotope analysis ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$)

Lipids, being depleted in ^{13}C relative to proteins and carbohydrates, can introduce bias into $\delta^{13}\text{C}$ measurements and are therefore removed prior to analysis (Post et al. 2007). Freeze-dried yolk and fish muscle samples had lipids removed using a 2:1 chloroform:methanol soak and rinse before being air dried in a fume hood. Samples of yolk, albumen, and red blood cells were pulverized into a fine powder and weighed into Elemental Microanalysis Ltd. tin capsules (1 mg) then sent to the National Hydrology Research Centre (NHRC) Stable Isotope Laboratory in Saskatoon, SK. At the NHRC lab, carbon and nitrogen isotope ratios were measured using an isotope ratio mass spectrometer (IRMS). Isotope ratios are reported in per mil (‰) relative to Vienna–Peedee Belemnite (VPDB) for $\delta^{13}\text{C}$ and atmospheric N_2 (AIR) for $\delta^{15}\text{N}$. Measurement error based on replicate measurements of in-house organic standards was estimated to be $\pm 0.15\text{‰}$ and $\pm 0.11\text{‰}$ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively.

2.4 Dietary niche analysis

Trophic position (TP) and the relative contribution of benthic versus pelagic carbon (α_R) were estimated using the *trps* package in R (Hlina 2025), which implements the two-baseline Bayesian model of Heuvel et al. (2025). This formulation extends the single-baseline model of Post (2002) by explicitly incorporating isotopic variation between benthic and pelagic baselines, as motivated by Vander Zanden and Vadeboncoeur (2002).

2.4.1 Relative benthic carbon use

Benthic carbon use (α) can be computed according to Post (2002):

$$\alpha = \frac{\delta^{13}\text{C}_C - \delta^{13}\text{C}_P}{\delta^{13}\text{C}_B - \delta^{13}\text{C}_P}$$

where $\delta^{13}\text{C}_C$ is the $\delta^{13}\text{C}$ of the consumer (cormorant chick), $\delta^{13}\text{C}_P$ is the mean $\delta^{13}\text{C}$ of the pelagic baseline and $\delta^{13}\text{C}_B$ is the mean $\delta^{13}\text{C}$ of the benthic baseline. Dreissenid mussels were used as the pelagic endmember, and oligochaete worms were used as the benthic endmember. Basin-specific baseline $\delta^{13}\text{C}$ values were obtained from Heuvel et al. (2025). α is the proportion of carbon that comes from each source and should be bound by 0 and 1. Because α may fall outside 0–1 when endmembers do not span the system's full $\delta^{13}\text{C}$ range, I calculated relative benthic carbon use (α_R) according to Heuvel et al. (2025):

$$\alpha_R = \frac{\alpha_C - \alpha_{min}}{\alpha_{max} - \alpha_{min}}$$

where α_{min} and α_{max} are the 1st and 99th percentiles of α from the same basin in which the consumer was collected. This preserves variability and avoids truncation of values that are less than 0 or greater than 1 for α .

2.4.2 Trophic position

I estimated chick trophic position (TP) with the two-baseline Bayesian framework in *trps*, pairing each chick to basin-specific lower-trophic baselines from Heuvel et al. (2025):

$$TP = [(\lambda_B \times \alpha_R) + (\lambda_P \times [1 - \alpha_R])] + \frac{\delta^{15}\text{N}_C - ((\delta^{15}\text{N}_B \times \alpha_R) + (\delta^{15}\text{N}_P \times [1 - \alpha_R]))}{\Delta N}$$

where λ_B and λ_P are the trophic positions of the organisms used as benthic and pelagic baselines respectively, $\delta^{15}\text{N}_C$ is the $\delta^{15}\text{N}$ of the cormorant chick, $\delta^{15}\text{N}_B$ and $\delta^{15}\text{N}_P$ are the mean benthic and pelagic baseline values (matched to the chick's basin to avoid spatial bias), α_R from the previous equation, and ΔN being the trophic enrichment factor.

Dreissenid mussels (filter feeders that integrate pelagic seston) were used as the pelagic pathway baseline and oligochaete worms were used as the benthic pathway baseline.

Following Heuvel et al. (2025), they were assigned trophic positions (λ) of 2.0 (λ_P) and 2.5 (λ_B) respectively, consistent with their typical roles as primary consumers. I retained $\lambda_B = 2.5$ to reflect that deposit-feeding organisms can incorporate detritus that may occupy an intermediate trophic position; for example, microbe-colonized plant detritus can have a TP of ~ 1.65 and consumers feeding on it a TP of ~ 2.79 (Steffan et al. 2017). To avoid spatial bias, mean $\delta^{15}\text{N}$ baseline values were always taken from the chick's own basin (obtained from Heuvel et al. 2025). The trophic enrichment factor was centered at 3.4‰ per trophic step (Post 2002), implemented as the default informed prior in *trps*. This formulation differs from the traditional single-baseline Post (2002) approach by explicitly mixing baselines from benthic and pelagic sources, yielding TP estimates that integrate both carbon source and nitrogen baseline structure. Compared with plug-in calculations, the *trps* two-baseline Bayesian model propagates sources of uncertainty, yielding posterior means with 95% credible intervals rather than single point estimates.

2.5 Statistical analysis

All statistical analyses were performed, and graphics produced using RStudio software version 2025.09.0 (R Core Team 2022). Normality was assessed by Shapiro–Wilk tests and Q–Q plots, and skew assessed by skewness coefficients. Data were log transformed to satisfy normality when possible and then back transformed on the original scale. *t* procedures were treated as robust to non-normality when each group had $n \geq 15$ (Watkins 2019); when assumptions could not be met for *t* procedures, a two-sample or paired Monte Carlo permutation test (9,999) was performed using the *coin* package (Hothorn et al. 2008).

3 Results

3.1 Blood mercury concentrations

Total mercury (THg) and methylmercury (MeHg) concentrations measured in chick red blood cells were significantly different between sites (Figure 3). Concentrations of THg were significantly higher in chicks from Middle Sister Island in the west basin ($n = 15$; mean \pm SD = $1,867.5 \pm 301.4$ ng/g d.w.) than from Mohawk Island in the east basin ($n = 39$; 660.8 ± 209.7 ng/g d.w.; $t(19.5) = 14.24$, $p < 0.001$). The distribution of total mercury concentration in red blood cells was approximately normal for the west basin ($W = 0.923$, $p = 0.216$; skew = 0.13) but was slightly right skewed and deviated from normality for the east basin ($W = 0.938$, $p = 0.033$; skew = 0.35).

Methylmercury in chick red blood cells was significantly higher in the west basin ($n = 12$; 1688.3 ± 457.3 ng/g d.w.) compared to the east basin ($n = 34$; 542.4 ± 178.5 ng/g d.w.; $t(18.55) = 10.12$, $p < 0.001$). The percentage of MeHg (% MeHg) in red blood cells was $89.0 \pm 23.2\%$ in the west ($n = 12$) and $80.4 \pm 10.8\%$ in the east ($n = 34$); a two-sample permutation test indicated no difference between islands ($Z = -1.68$, $p = 0.095$) in the % of THg that was MeHg. The distribution of methylmercury concentration in red blood cells was significantly right-skewed at the east site ($W = 0.916$, $p = 0.012$; skew = 0.56). Although the west site exhibited negative skew (skew = -0.92), the Shapiro–Wilk test did not indicate a statistically significant deviation from normality ($W = 0.908$, $p = 0.202$).

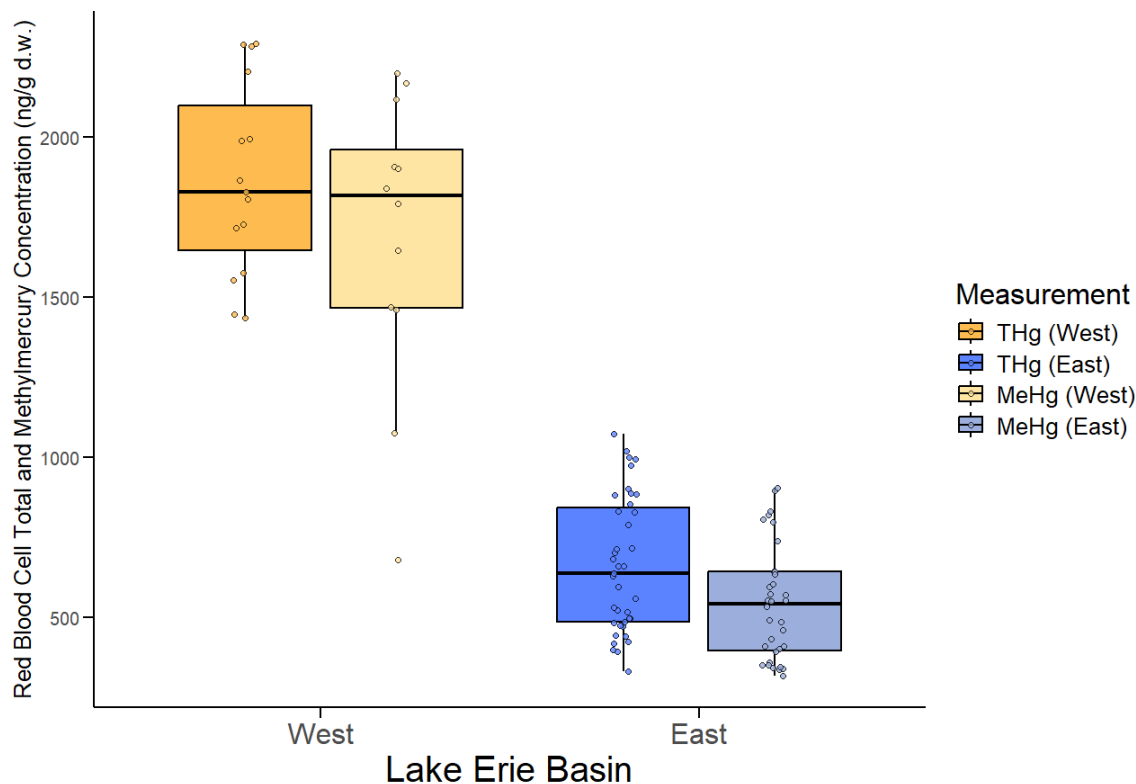


Figure 3. Box plot illustrating total mercury (THg) and methylmercury (MeHg) concentrations in ng/g d.w. measured in cormorant chick red blood cell samples from two islands: Middle Sister Island (west; THg n=15; MeHg n=12) and Mohawk Island (east; THg n=39; MeHg n=34) in 2022–2024. The horizontal lines within each box represent the median values, the box spans the interquartile range (IQR), and the whiskers extend to 1.5 * IQR. Dots indicate individual observations.

3.2 Chick mass versus blood mercury concentrations

East basin chicks were significantly heavier ($n = 27$; mean \pm SD = 1,562.9 \pm 198.4 g) than west basin chicks on the date that they were sampled ($n = 7$; 1,350.0 \pm 138.4 g; $t(13.2) = 3.29$, $p < 0.01$). Simple linear regressions for mass versus blood mercury concentrations showed no relationship between blood mercury concentrations (THg or MeHg) and chick mass at either site; tests confirmed normality and homoscedasticity; no influential cases were detected; all p-values exceeded 0.05 and all confidence intervals

included 0. In the west, the THg slope was 0.171 g per ng/g d.w. (95% CI: -0.229 to 0.571, $p = 0.321$) and the MeHg slope was 0.014 (95% CI: -0.280 to 0.307, $p = 0.910$). In the east, the THg slope was 0.158 (95% CI: -0.239 to 0.555, $p = 0.421$) and the MeHg slope was 0.273 (95% CI: -0.149 to 0.695, $p = 0.194$).

3.3 Egg mercury concentrations

Albumen THg did not differ significantly between east (Figure 4; $n = 47$; mean \pm SD = 1318.8 ± 567.4 ng/g d.w.) and west basin eggs ($n = 33$; 1296.2 ± 464.2 ng/g d.w.; $Z = 0.19$, $p = 0.857$). Yolk THg also showed no difference between east (139.3 ± 85.0 ng/g d.w.) and west basin eggs (122.3 ± 42.7 ng/g d.w.; $Z = 1.06$, $p = 0.299$). Albumen MeHg was similar between east (1051.2 ± 524.0 ng/g d.w.) and west basin eggs (1038.6 ± 361.0 ng/g d.w.; $Z = 0.12$, $p = 0.910$). Yolk MeHg likewise did not differ between east (49.9 ± 35.2 ng/g d.w.) and west basin eggs (47.2 ± 28.6 ng/g d.w.; $Z = 0.36$, $p = 0.725$).

Within each basin, albumen concentrations were far higher than yolk for both THg and MeHg. In east basin eggs, albumen THg (1318.8 ± 567.4 ng/g d.w.) greatly exceeded yolk THg ($n = 47$; 139.3 ± 85.0 ng/g d.w.; paired permutation test: $Z = -6.31$, $p < 0.001$). Likewise, albumen MeHg (1051.2 ± 524.0 ng/g d.w.) was higher than yolk MeHg in east basin eggs ($n = 47$; 49.9 ± 35.2 ng/g d.w.; $Z = -6.15$, $p < 0.001$). In west basin eggs, albumen THg (1296.2 ± 464.0 ng/g d.w.) exceeded yolk THg ($n = 33$; 122.3 ± 42.7 ng/g d.w.; $Z = -5.38$, $p < 0.001$). Albumen MeHg (1038.6 ± 361.3 ng/g d.w.) was likewise greater than yolk MeHg in west basin eggs ($n = 33$; 47.2 ± 28.6 ng/g d.w.; $Z = -5.43$, $p < 0.001$). On each island, albumen % MeHg was far higher than yolk % MeHg. In the east, a paired permutation test indicated albumen % MeHg exceeded yolk % MeHg ($Z = -6.33$, $p < 0.001$); in the west, the corresponding difference was likewise highly significant ($Z = -5.39$, $p < 0.001$).

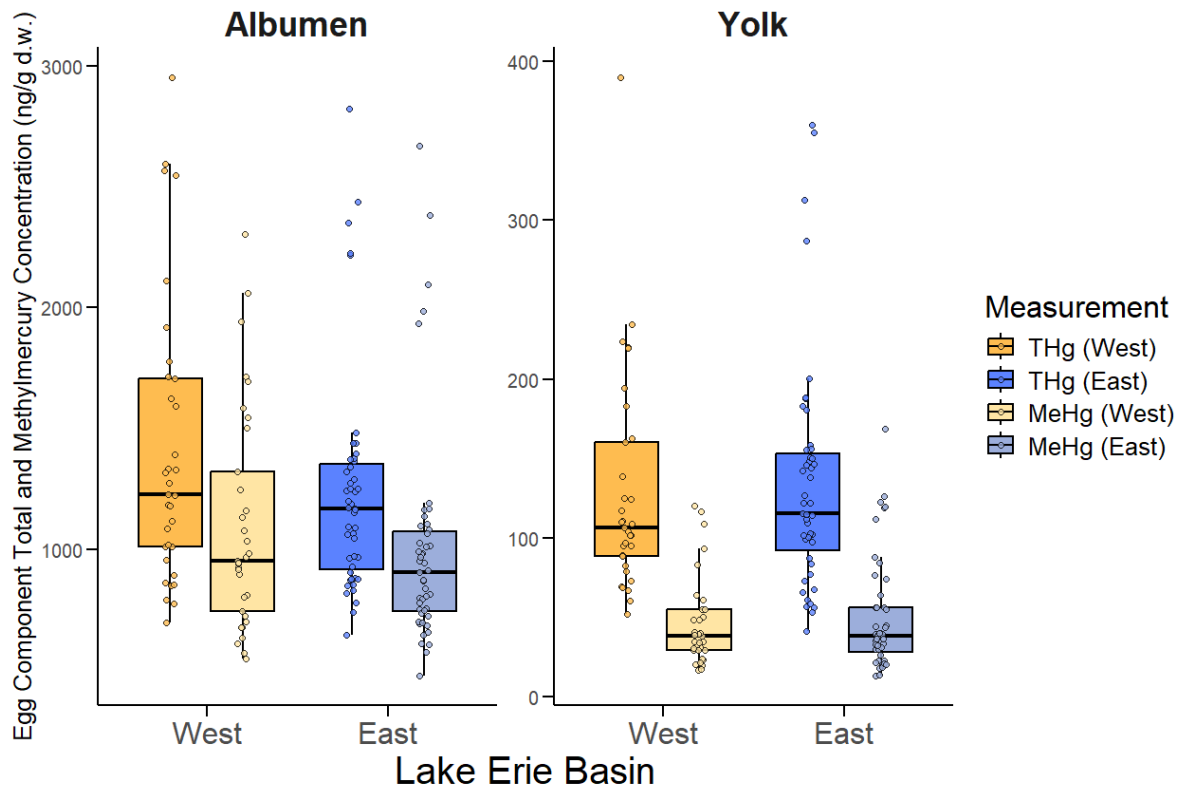


Figure 4. Left: Box plot illustrating total mercury (THg) and methylmercury (MeHg) concentrations in ng/g d.w. measured in cormorant albumen from two Lake Erie sites: Middle Sister Island (west; n=33) and Mohawk Island (east; n=47) in 2024. Right: Box plot illustrating total THg and MeHg concentrations in ng/g d.w. measured in cormorant yolk from west (n=33) and east (n=47) sites in 2024. The horizontal lines within each box represent the median values, the box spans the interquartile range (IQR), and the whiskers extend to 1.5 * IQR. Dots indicate individual observations.

3.4 Feather mercury

West basin chicks had significantly higher feather mercury concentrations than east basin chicks in both feather types (Figure 5). For P10 feathers, THg was elevated in the west ($n = 12$; mean \pm SD = 4417.1 ± 1346.6 ng/g d.w.) relative to the east ($n = 15$; 1417.7 ± 274.5 ng/g d.w.; two-sample permutation test: $Z = -4.39$, $p < 0.001$). P10 MeHg followed the same pattern (west: 4263.5 ± 769.8 ng/g d.w. vs east: 1628.2 ± 406.3 ng/g d.w.; $Z = -4.67$, $p < 0.001$). Similarly, for R1 feathers, THg was higher in the west ($n = 20$; 3956.1 ± 817.9 ng/g d.w.) than in the east ($n = 14$; 1331.4 ± 259.9 ng/g d.w.; $Z = -5.16$, $p < 0.001$), and MeHg showed the same trend (west: 3667.8 ± 998.8 ng/g d.w. vs east: 1545.7 ± 236.3 ng/g d.w.; $Z = -4.64$, $p < 0.001$).

Paired tests indicated no significant differences between feather types. For THg, R1 and P10 feathers did not differ ($n = 26$; $Z = 0.84$, $p = 0.715$). MeHg likewise showed no difference between R1 and P10 ($n = 26$; $Z = 1.32$, $p = 0.199$).

Percent methylmercury also differed between islands for one of the two feather types. For R1 feathers, the proportion of mercury present as MeHg was somewhat and statistically significantly lower in the west (mean \pm SD = $92.2 \pm 13.7\%$) than in the east ($117.4 \pm 10.7\%$; $Z = -4.06$, $p < 0.001$). In contrast, P10 feathers showed no significant difference in % MeHg between colonies (west: $101.5 \pm 22.3\%$; east: $115.3 \pm 18.9\%$; $Z = -1.68$, $p = 0.094$). Within colonies, paired permutation tests indicated no significant differences between feather types. In the west basin, R1 and P10 feathers showed comparable methylation percentages ($n = 12$; $Z = -0.81$, $p = 0.410$), as did those from the east basin ($n = 14$; $Z = 0.37$, $p = 0.725$).



Figure 5. Left: Box plot illustrating total mercury (THg) and methylmercury (MeHg) concentrations in ng/g d.w. measured in cormorant primary feathers (P10) from two Lake Erie sites: Middle Sister Island (west; n = 12) and Mohawk Island (east; n = 15) in 2024. Right: Box plot illustrating total THg and MeHg concentrations in ng/g d.w. measured in cormorant rectrix feathers (R1) from west (n = 20) and east (n = 14) sites in 2024. The horizontal lines within each box represent the median values, the box spans the interquartile range (IQR), and the whiskers extend to 1.5 * IQR. Dots indicate individual observations.

3.5 Feather versus blood mercury

Paired permutation tests confirmed that feather mercury concentrations were significantly higher than blood concentrations. P10 feather THg was greater than red blood cell THg in both colonies: in the west basin ($n = 7$; $Z = -2.42$, $p < 0.05$) and in the east basin ($n = 15$; $Z = -3.72$, $p < 0.001$). Methylmercury showed the same pattern (west: $n = 7$; $Z = -2.42$, $p < 0.05$; east: $n = 15$; $Z = -3.72$, $p < 0.001$). For R1 feathers, THg concentrations similarly exceeded blood for chicks in the west ($n = 7$; $Z = -2.42$, $p < 0.05$) and east basins ($n = 14$; $Z = -3.72$, $p < 0.001$), and MeHg followed the same trend (west: $n = 7$; $Z = -2.42$, $p < 0.05$; east: $n = 14$; $Z = -3.72$, $p < 0.001$).

In the east basin, red blood cell THg concentrations were positively correlated with feather THg (Figure 6; $n = 14$, Pearson's $r = 0.64$ for R1; $n = 15$, $r = 0.617$ for P10). Using simple linear regression, each 1,000 ng/g d.w. increase in R1 THg corresponded to an estimated 444 ng/g d.w. increase in blood THg ($R^2 = 0.41$, $p < 0.05$). The P10 model was similar with a slope of 408 ng/g d.w. per 1,000 ng/g d.w. ($R^2 = 0.381$, $p < 0.05$).

Relationships with red blood cell MeHg were weaker for MeHg in the R1 feather ($r = 0.488$) and stronger for MeHg in the P10 feather (Figure 7; $r = 0.726$). Regression models indicated that methylmercury in the P10 feather explained 52.7% of the variance in red blood cell MeHg ($R^2 = 0.527$; slope = 251 ng/g d.w. per 1,000 ng/g d.w., $p < 0.005$), whereas methylmercury in the R1 feather explained 23.8% ($R^2 = 0.238$; slope = 288 ng/g d.w. per 1,000 ng/g d.w., $p = 0.0769$).

In the west basin, feather–red blood cell mercury relationships were stronger for THg. Red blood cell THg was highly correlated with feather THg (Figure 6; $n = 7$, $r = 0.767$ for R1; $n = 7$, $r = 0.82$ for P10). Regression slopes were consistent across feather types: 282 ng/g d.w. per 1,000 ng/g d.w. for R1 and 277 ng/g d.w. per 1,000 ng/g d.w. for P10, with models explaining 59–67% of the variance ($R^2 = 0.589$ for R1, $p < 0.05$; $R^2 = 0.673$ for P10, $p < 0.05$). Red blood cell MeHg was correlated with MeHg in both R1 and P10 feathers (Figure 7; $r = 0.685$ and $r = 0.694$, respectively). Regression slopes were 305 ng/g d.w. per 1,000 ng/g d.w. for R1 ($R^2 = 0.470$, $p = 0.0892$) and 443 ng/g d.w. per 1,000 ng/g d.w. for P10 ($R^2 = 0.481$, $p = 0.0839$), each explaining about 47–48% of the variance.

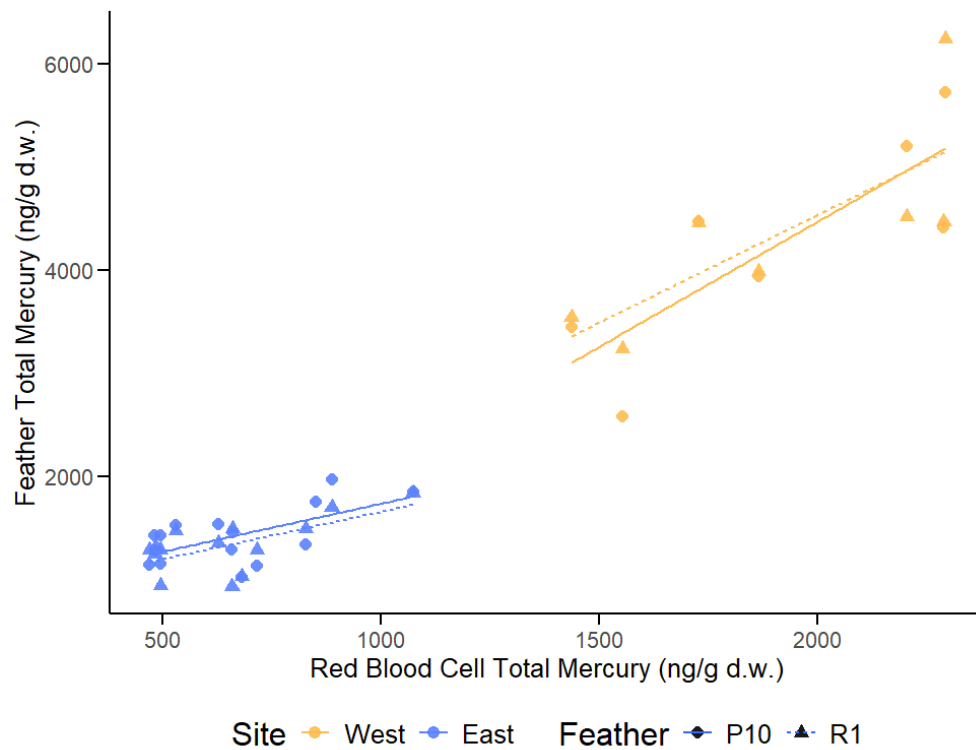


Figure 6. Scatterplot illustrating the relationship between red blood cell total mercury (THg; ng/g d.w.) and feather THg (ng/g d.w.) in Double-crested Cormorant chicks from two Lake Erie colonies: Middle Sister Island (west; n = 7) and Mohawk Island (east; n = 15 for P10; n = 14 for R1) in 2024. Individual points represent chicks, coloured by site; solid regression lines denote P10 feathers and dotted lines denote R1 feathers.

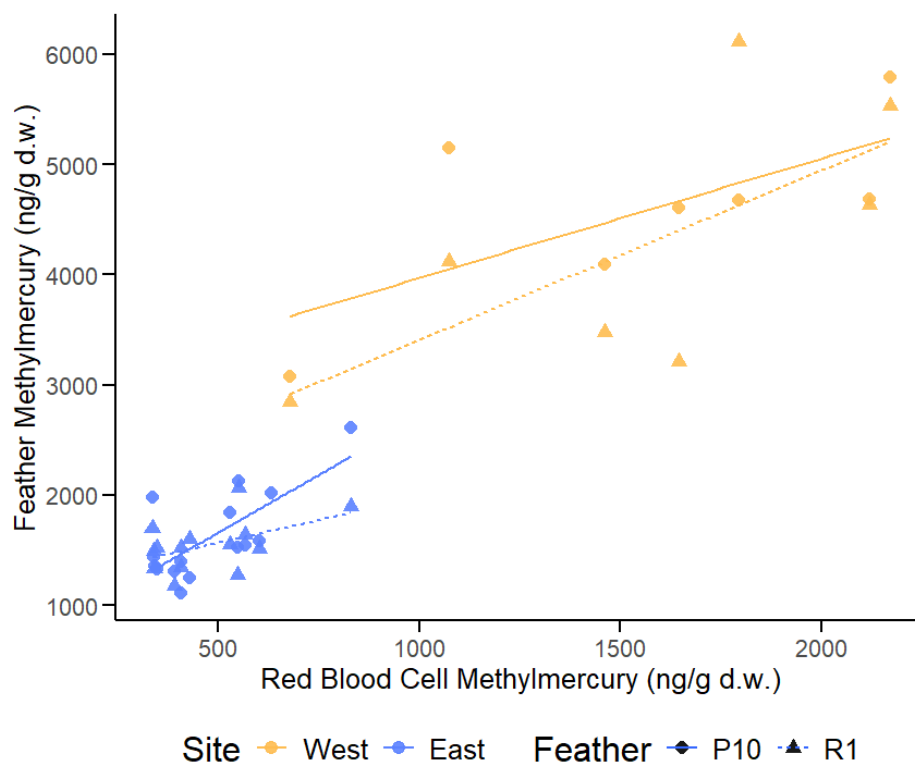


Figure 7. Scatterplot illustrating the relationship between red blood cell methylmercury (MeHg; ng/g d.w.) and feather MeHg (ng/g d.w.) in Double-crested Cormorant chicks from two Lake Erie colonies: Middle Sister Island (west; $n = 7$) and Mohawk Island (east; $n = 15$ for P10; $n = 14$ for R1) in 2024. Individual points represent chicks, coloured by site; solid regression lines denote P10 feathers and dotted lines denote R1 feathers.

3.6 Relative benthic carbon use

Basin-specific relative benthic carbon use (α_R) did not significantly differ by site (Figure 8; $t(23.51) = 1.30, p = 0.207$). Mean \pm SD α_R was 0.553 ± 0.237 for the east basin chicks ($n = 39$) and 0.453 ± 0.260 for the west basin chicks ($n = 15$). There was an estimated mean difference (east – west) of 0.100 α_R units (95% CI: -0.059 – 0.260); the confidence interval overlaps zero and does not support a reliable between-basin difference.

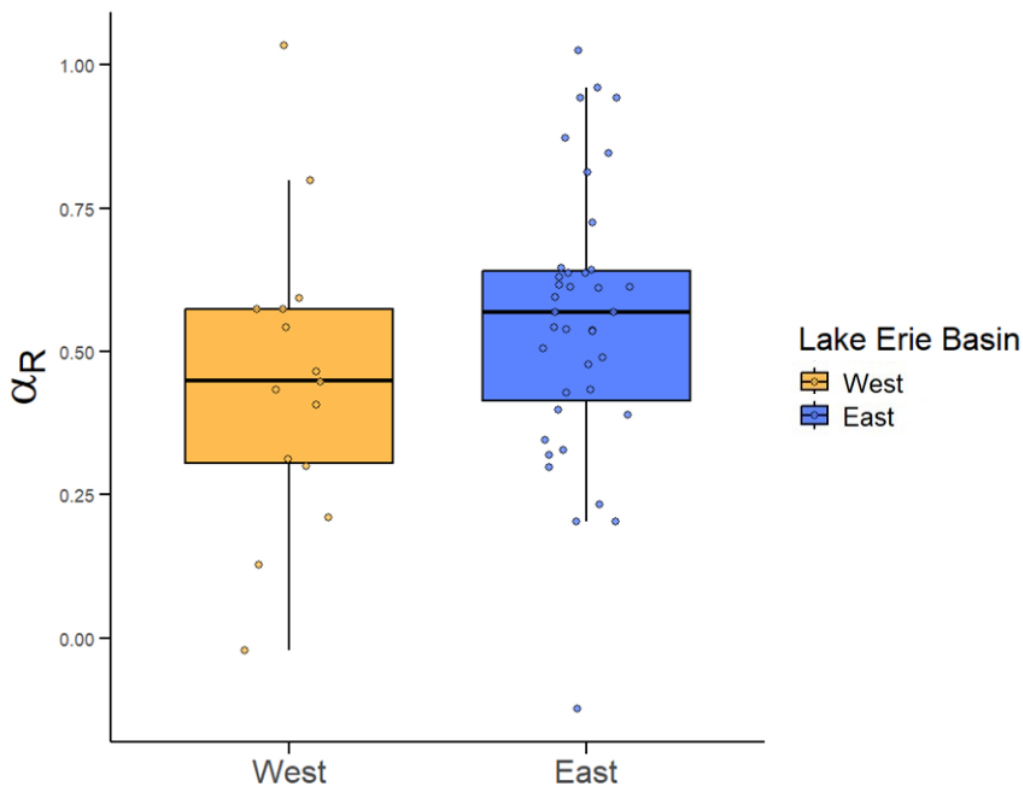


Figure 8. Box plot illustrating calculated α_R (relative benthic carbon use) from $\delta^{13}\text{C}$ of cormorant chick red blood cell samples at two Lake Erie sites: Middle Sister Island (west; n=15) and Mohawk Island (east; n=39) in 2022–2024. The horizontal lines within each box represent the median values, the box spans the interquartile range (IQR), and the whiskers extend to $1.5 * \text{IQR}$. Dots indicate individual observations. Calculations were performed using the `trps` package in RStudio. Dreissenids and oligochaetes were used as isotopic endmembers (values obtained from Heuvel et al. 2025).

3.7 Chick trophic position

West basin chicks occupied a higher mean trophic position ($n = 15$; posterior mean \pm SD = 4.30 ± 0.18 ; 95% CrI: 4.00–4.68) than east basin chicks (Figure 9; $n=39$; 3.78 ± 0.13 ; 95% CrI: 3.54–4.05). The posterior difference (west – east) was 0.52 (95% CrI: 0.12–0.98), and there was a 99.5% posterior probability that trophic position was higher at the west site compared to the east.

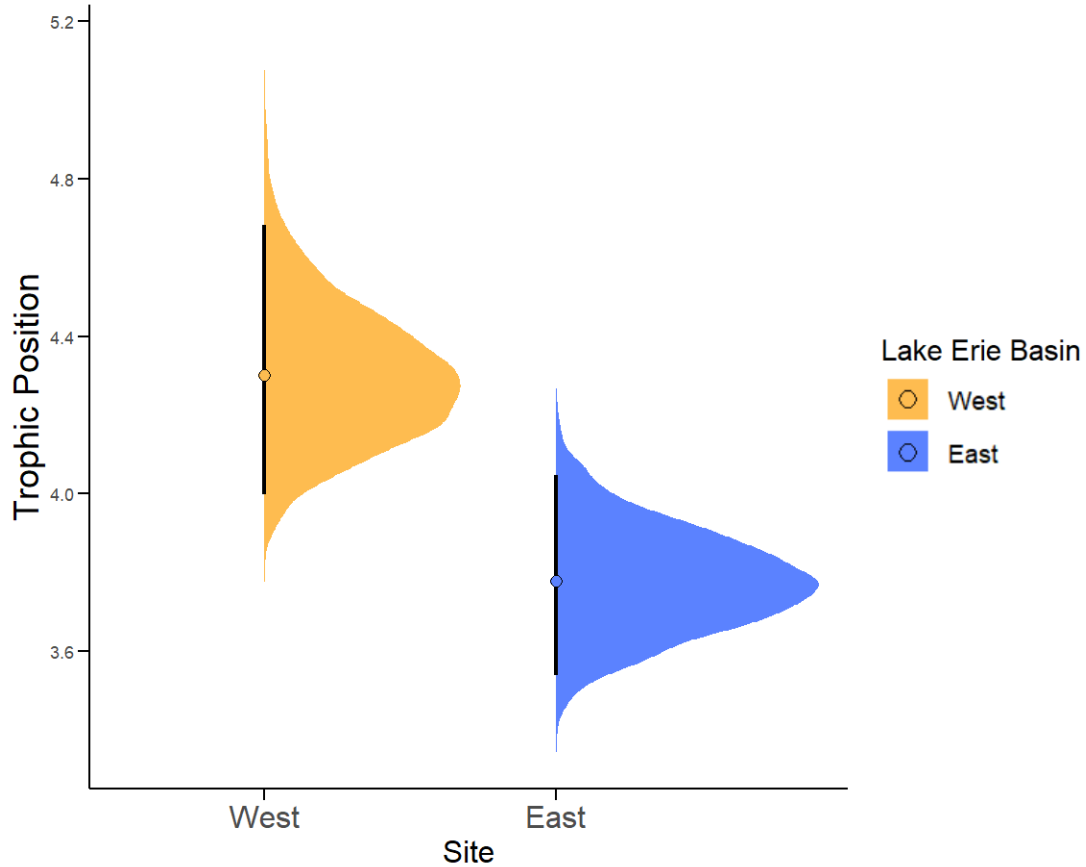


Figure 9. Posterior trophic position of cormorant chicks at two Lake Erie sites: Middle Sister Island (west; $n = 15$) and Mohawk Island (east; $n = 39$), 2022–2024. Half-eye density plots show the posterior distribution of each site’s mean trophic position; open points and vertical bars denote the posterior mean and 95% credible interval. Trophic position was estimated with Stan (brms) using the *trps* package two-baseline framework with basin-specific baselines (values from Heuvel et al. 2025) in RStudio.

3.8 Trophic position versus blood mercury concentrations

Spearman correlations revealed basin-specific relationships between trophic position and mercury concentrations in chick red blood cells (Figure 10). In west basin chicks, no association was detected for either THg ($n = 15$; $\rho = 0.025$, 95% CI: -0.536 – 0.574 , $p = 0.930$) or MeHg ($n = 12$; $\rho = -0.014$, 95% CI: -0.724 – 0.577 , $p = 0.966$). In contrast, statistically significant positive correlations were observed in the east basin for both THg and MeHg versus trophic position (THg: $n = 39$; $\rho = 0.793$, 95% CI: 0.626 – 0.882 , $p < 0.001$; MeHg: $n = 34$; $\rho = 0.613$, 95% CI: 0.297 – 0.820 , $p < 0.001$).

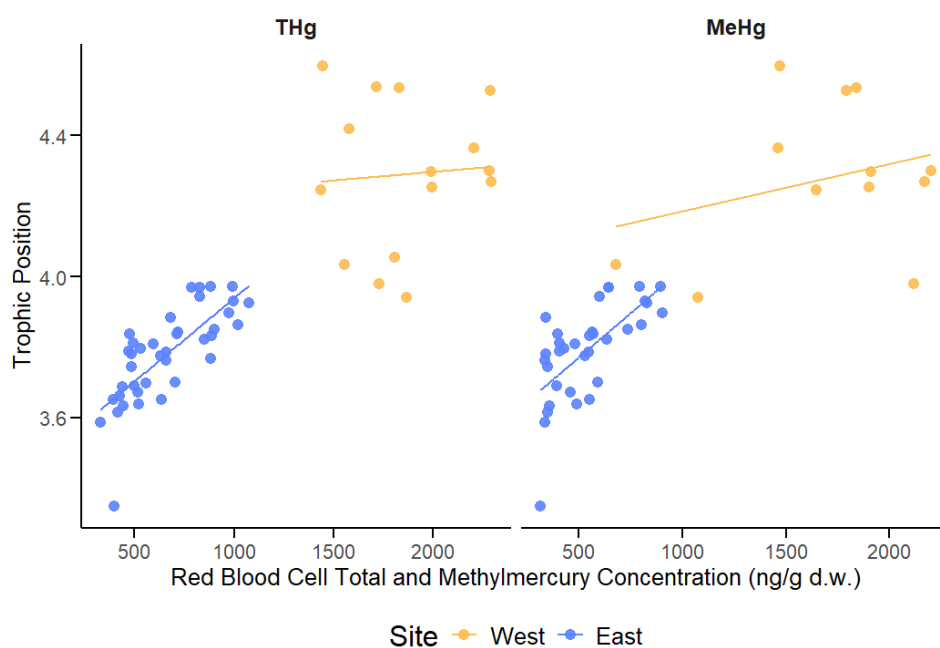


Figure 10. Left: Scatterplot illustrating the relationship between trophic position and red blood cell total mercury (THg; ng/g d.w.) in Double-crested Cormorant chicks from two Lake Erie colonies in 2022–2024: Middle Sister Island (west; $n = 15$) and Mohawk Island (east; $n = 39$). Right: Scatterplot illustrating the relationship between trophic position and red blood cell methylmercury (MeHg; ng/g d.w.) for the same chicks (west: $n = 12$; east: $n = 34$). Points represent individual chicks coloured by site, and solid lines show linear regressions for visual interpretation.

3.9 Dietary mercury concentrations

Although regurgitated fish were obtained from chicks at both sites (Table 1), the low number of samples per fish species precluded statistical analysis of dietary composition and dietary mercury by basin. Round Goby tissue from the west ($n = 1$) had a THg concentration of 967.1 ng/g d.w. and a MeHg concentration of 954.1 ng/g d.w., whereas east-basin gobies ($n = 7$) averaged (mean \pm SD) 112.7 ± 30.7 ng/g d.w. for THg and 100.5 ± 26.3 ng/g d.w. for MeHg. Yellow Perch showed a similar but smaller difference: west ($n = 1$) THg = 276.8 ng/g d.w. and MeHg = 228.1 ng/g d.w. versus east ($n = 1$) THg = 175.3 ng/g d.w. and MeHg = 174.0 ng/g d.w.

Table 1. Summary of mean \pm SD fork length (FL), $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable-isotope values, total mercury (THg) concentrations in muscle tissue of fish sampled at West and East sites of Lake Erie (May–June 2024).

Muscle tissues were dissected from thawed specimens (frozen at $-20\text{ }^{\circ}\text{C}$), freeze-dried, and homogenized prior to analysis. Stable-isotope ratios ($\delta^{13}\text{C}$ relative to VPDB; $\delta^{15}\text{N}$ relative to AIR) were determined on lipid-extracted samples. THg was quantified on a dry-weight (d.w.) basis using a direct mercury analyzer. Sample size (n) per species and site combination is indicated; missing SD values reflect single observations.

| Species | Site | n | FL | | $\delta^{13}\text{C}$ | $\delta^{13}\text{C}$ | $\delta^{15}\text{N}$ | $\delta^{15}\text{N}$ | THg | THg |
|-----------------|------|---|-----------|------------|-----------------------|-----------------------|-----------------------|-----------------------|-------------|-----------|
| | | | Mean (cm) | FL SD (cm) | Mean (‰) | SD (‰) | Mean (‰) | SD (‰) | Mean (ng/g) | SD (ng/g) |
| Alewife | East | 6 | 11.3 | 1.1 | -23.2 | 0.5 | 17.2 | 1.8 | 151.4 | 84.5 |
| Pumpkinseed | East | 8 | 7.6 | 1.5 | -21.5 | 0.2 | 7.9 | 0.5 | 1827.0 | 353.7 |
| Round Goby | East | 7 | 8.5 | 1.7 | -23.5 | 1.0 | 15.7 | 0.5 | 112.7 | 30.7 |
| Round Goby | West | 1 | 8.0 | — | -17.1 | — | 14.8 | — | 967.1 | — |
| Smallmouth Bass | West | 1 | 14.4 | — | -21.3 | — | 14.7 | — | 457.0 | — |
| Trout-perch | East | 3 | 5.3 | 0.3 | -23.4 | 1.7 | 15.7 | 1.8 | 135.0 | 114.4 |
| Walleye | East | 2 | 23.5 | 0.7 | -21.4 | 1.5 | 18.3 | 0.7 | 718.9 | 346.8 |
| Walleye | West | 3 | 16.9 | 5.1 | -23.1 | 2.1 | 16.4 | 0.9 | 428.0 | 362.4 |
| White Perch | West | 1 | 14.5 | — | -23.3 | — | 16.9 | — | 365.7 | — |
| Yellow Perch | East | 1 | 7.4 | — | -23.3 | — | 15.9 | — | 175.3 | — |
| Yellow Perch | West | 1 | 15.6 | — | -21.0 | — | 15.6 | — | 276.8 | — |

4 Discussion

4.1 Chick blood mercury

Mercury (Hg) levels in the red blood cells of Double-crested Cormorants showed a clear spatial pattern across Lake Erie: chicks from Middle Sister Island in the west basin had roughly three times more mercury than those from Mohawk Island in the east. On average, west basin chicks had a red blood cell total mercury (THg) concentration of 1,868 ng/g d.w. (dry weight), whereas east basin chicks measured just 661 ng/g d.w. Using the whole blood wet weight conversion provided in Ackerman et al. (2024; Eq. 7), these red blood cell dry weight values correspond to approximately 0.41 $\mu\text{g/g}$ w.w. and 0.14 $\mu\text{g/g}$ w.w., respectively, although there is some uncertainty in this conversion related to moisture content assumptions. This pronounced disparity highlights markedly different contaminant exposures at the two colonies.

Literature on Double-crested Cormorant blood mercury concentration is scarce. A notable study from arid-land reservoirs in south-central New Mexico reported mercury levels comparable to west basin Lake Erie chicks. Nestlings at Caballo Reservoir averaged 0.36 $\mu\text{g/g}$ w.w. at 7–10 days post-hatch and 0.39 $\mu\text{g/g}$ w.w. at 17–22 days; Elephant Butte Reservoir chicks showed similar concentrations of 0.36 $\mu\text{g/g}$ and 0.34 $\mu\text{g/g}$ for the same age groups (Caldwell et al. 1999). These New Mexico nestling blood values occurred in a part of the Rio Grande where water-column MeHg was somewhat elevated but temporally dynamic. Within the reservoir, unfiltered surface-water MeHg in Elephant Butte reached about 0.26 ng/L following autumn turnover, after being below detection (<0.018 ng/L) during summer stratification (Canavan et al. 2000). In the Elephant Butte tailrace, MeHg rose from 0.149 to 1.144 ng/L during late-summer stratification, then declined to 0.420 ng/L after autumn turnover. In comparison, Starr et al. (2024) reported unfiltered MeHg concentrations in western Lake Erie surface water of 0.29 pM (~0.06 ng/L) near the Detroit River and 0.63 pM (~0.14 ng/L) at the Maumee River mouth during late summer and early fall.

Despite much lower methylmercury in the water column of Lake Erie, chicks in the west basin of Lake Erie had higher mercury burdens than those in the Rio Grande. Mercury

methylation is often concentrated near active oxic/anoxic boundaries at the sediment–water interface and can shift seasonally with stratification and turnover, so discrete surface-water snapshots can miss the habitats or time windows that drive prey contamination (Millard et al. 2023). This disconnect highlights the value of monitoring high trophic level organisms, which integrate localized methylation and food-web transfer that may not be apparent from surface-water mercury concentrations alone.

Measuring mercury in chick blood gives a snapshot of recent dietary exposure as it reflects short-term accumulation. Previous studies have shown that methylmercury is eliminated from the blood of chicks on the scale of days to weeks, much faster than in adults (Evers et al. 2008). Although the specific MeHg half-life for cormorant chicks is not yet established, related studies offer useful comparisons. For instance, in chicks undergoing feather molt, Common Loons (*Gavia immer*) exhibit a blood MeHg half-life of about three days (Fournier et al. 2002), while Cory’s Shearwaters (*Calonectris borealis*) have a slightly longer half-life of around five to six days (Monteiro and Furness 2001). Thus, chick blood mercury concentrations can respond quickly to changes in dietary intake, and any sustained elevation must be maintained by continual mercury ingestion.

At both Lake Erie colonies, methylmercury (MeHg) represented the majority (approximately 80–90%) of total mercury detected in chick red blood cells, confirming that the mercury accumulation was predominantly in a bioavailable and toxic form. This finding aligns with previously reported trends for piscivorous bird species (Fournier et al. 2002; Álvarez et al. 2013). The predominance of MeHg in chick blood indicates that observed differences between colonies largely reflect variation in the bioavailable, diet-derived fraction of mercury rather than inorganic forms. Because red blood cell mercury represents the fraction actively circulating and available for uptake into tissues (Giblin and Massaro 1975), these concentrations provide a meaningful measure of recent exposure during the chick-rearing period, forming a basis for interpreting their toxicological relevance.

To gauge the ecological significance of these concentrations, it is important to compare them against avian toxicity benchmarks. Although higher mercury burdens in the west basin chicks (1,868 ng/g d.w. or 0.41 $\mu\text{g/g}$ w.w.) are notable, they remain below commonly referenced adult blood toxicity thresholds. Ackerman et al. (2016) classify adult whole blood total mercury concentrations less than 1.0 $\mu\text{g/g}$ w.w. as low risk and 1.0 to 3.0 $\mu\text{g/g}$ w.w. as moderate risk for adults. These designations are useful for initial screening; however, juveniles are more sensitive to mercury toxicity than both adults and eggs in almost all biological effect categories examined (Ackerman et al. 2024).

More recent work from Ackerman et al. (2024) modeled how mercury affected a combined survival-and-reproduction endpoint for eggs, juveniles, and adults, and based their recommended toxicity reference values on juveniles because they were the most sensitive to mercury. In this framework, injury categories were defined from effective concentrations (EC_x) that produce x percent declines in combined survival and reproduction endpoints: 0.09 $\mu\text{g/g}$ w.w. (EC₁, low injury), 0.6 $\mu\text{g/g}$ w.w. (EC₅, moderate injury), 1.3 $\mu\text{g/g}$ w.w. (EC₁₀, high injury), and 3.2 $\mu\text{g/g}$ w.w. (EC₂₀, severe injury; Ackerman et al. 2024). On this scale, all west basin cormorant chicks had blood mercury concentrations above the benchmark dose lower bound of 0.3 $\mu\text{g/g}$ w.w., which represents the lower 90 percent credible interval for the EC₅ estimate, and the three highest individuals reached 0.50 $\mu\text{g/g}$ w.w., just below the moderate injury value of 0.6 $\mu\text{g/g}$ w.w. Although these categories are described for the juvenile age class because the underlying models relate mercury exposure to juvenile survival and reproduction, the exposure metric itself was standardized to adult whole blood equivalent concentrations using egg, chick down, and adult tissues rather than juvenile blood. Therefore, the comparison between chick blood concentrations and the toxicity reference values should be interpreted as an approximate indication of risk, rather than as an exact toxicity threshold for this specific age class and tissue.

Even though these chicks are in lower risk categories, physiological effects from mercury exposure remain possible—particularly since this exposure occurs early in development. Mercury's strong oxidizing potential means that even moderate exposure can activate cellular stress pathways. Previous research has demonstrated a positive correlation

between blood mercury concentrations and oxidative stress gene expression in cormorants (Gibson et al. 2014). Specifically, female cormorants with elevated blood mercury levels showed increased activation of antioxidant defense mechanisms, suggesting mild oxidative stress even at exposures within the low-risk range ($<1.0 \mu\text{g/g}$ w.w.). Biochemical stresses could potentially divert energy away from essential processes such as growth or immune function in chicks. There is evidence across bird species that mercury can dampen immune responses even without clinical illness (Fallacara et al. 2011; Lewis et al. 2012). Therefore, any future comprehensive colony-level assessments should consider both overt and subtle sublethal effects of mercury exposure.

4.2 Chick weight and mercury relationship

In this study I used chick body mass as a proxy for age, on the assumption that chicks gain weight predictably as they develop. Chick masses from the west basin correspond to 18 to 27 days old, while east basin chicks correspond to 20 to 37 days old (Dunn 1975). No significant correlation between chick body weight and red blood cell mercury concentration was found within either basin. The absence of a clear within-site trend is biologically reasonable when considering how growth and mercury uptake interact.

Chicks undergo rapid growth which can dilute their body mercury concentrations. Chicks that grow faster (gain more mass) can exhibit lower mercury concentrations simply because the mercury is distributed in a larger body mass (Ackerman et al. 2011). On the other hand, chicks that receive more food (and thus grow well) also ingest more mercury with that food, potentially increasing their body mercury. These two processes (growth dilution versus intake) can counteract each other to some extent. It has been shown that chicks with the greatest mass gains tend to show the steepest declines in blood Hg over time, as their growth outpaces mercury intake (Ackerman et al. 2011). Chick mass can also vary based on feeding schedule, as a recently fed chick will weigh more than one with an empty stomach.

Individual differences in size could reflect slight age differences or differences in nutrition, but these do not appear to produce a consistent pattern in mercury burdens. A

well-fed or older chick might accumulate more mercury (through more food) but also achieve larger mass (more dilution), whereas a smaller or younger chick gets less food (less Hg) but also grows less (less dilution), resulting in comparable mercury concentrations. Thus, it is perhaps not surprising that no clear mass–mercury relationship emerged within sites.

4.3 Feather and blood mercury relationship

Feather and blood mercury comparisons revealed a pronounced between-site difference and underscored the role of growing plumage as a mercury sink. Both wing feathers (P10 primaries) and tail feathers (R1 rectrices) of west-basin cormorant chicks contained significantly higher mercury concentrations than those from the east basin (by roughly three-fold), mirroring the strong site disparity observed in blood Hg. Within individuals, P10 and R1 feathers did not differ in mercury content, indicating that either feather type captured the same exposure signal in these nestlings. However, feather mercury concentrations greatly exceeded red blood cell levels in both colonies, reflecting the well-known partitioning of methylmercury into developing plumage (Appelquist et al. 1984; Braune and Gaskin 1987). Accordingly, chicks with elevated blood mercury also had elevated feather mercury, a significant positive correlation that suggests feather values reliably track individual differences in circulating mercury during feather growth. Notably, the P10 feather's MeHg showed a stronger correlation with red blood cell MeHg than did R1's, likely due to slight timing offsets in feather development. Primary feathers are grown sequentially along the wing (P10 being one of the later primaries), while tail feathers emerge from the center; such differences in molt timing may cause temporal mismatches between feather Hg and blood Hg for feathers grown earlier (Bottini et al. 2021). In this case, P10's mercury content more closely reflected concurrent blood Hg at sampling, whereas R1 may have captured an earlier exposure period.

These patterns of feather–blood mercury partitioning align with the known physiological basis of mercury sequestration in feathers. Feathers are the principal route of MeHg elimination in birds, binding mercury in the keratin as they grow (Renedo et al. 2017). Once a feather is fully grown and the blood supply to it ceases, its mercury load is effectively locked, with mercury irreversibly bound to sulfhydryl groups of keratin; the

feather thus preserves a chemical record of the bird's mercury burden during the feather's growth interval (Renedo et al. 2017). By contrast, blood mercury is dynamic and can fluctuate with recent dietary intake and ongoing feather growth. While a chick's feathers are growing, a large fraction of absorbed MeHg is shunted into plumage, keeping blood concentration lower. Once feather growth is complete, blood mercury can rise sharply because the sink is gone (Condon and Cristol 2009). Experimental work on songbird nestlings demonstrated this clearly: juveniles had low blood mercury when many feathers were rapidly developing, but blood levels spiked to adult-like values after fledging when feathers no longer acted as a mercury sink (Condon and Cristol 2009). My findings are consistent with this dynamic. The fact that chick feathers contained far more mercury than blood indicates active depuration to plumage, and it explains why blood mercury in young birds may underestimate total exposure when feathers are still growing. A study of Double-crested Cormorant chicks by Caldwell et al. (1999) found that feather THg concentrations (on the order of 2–5 $\mu\text{g/g}$) were higher than in the blood ($\sim 0.36 \mu\text{g/g}$), and they noted no significant blood–feather correlation in chicks, which highlights the complexity of mercury redistribution during development. In my study, by 3–5 weeks old the chicks' feather growth was well underway but not entirely finished. In contrast to the findings of Caldwell et al. (1999), Hg concentrations in feathers were positively correlated to blood and reflected the post-hatch dietary uptake of chicks in my study. The significant feather–blood correlations I observed confirm that feather mercury is a robust integrator of recent body burden in these nestlings, even as absolute concentrations differ between tissue types. This is supported by large-scale comparisons which demonstrated that nestling feathers are effective proxies for adult mercury exposure and monitoring local mercury concentrations across Canada (Lavoie and Campbell 2018).

The feather mercury levels measured here (approximately 4.0 $\mu\text{g/g}$ THg in western Lake Erie chicks versus 1.4 $\mu\text{g/g}$ in eastern Lake Erie) fall within documented ranges for cormorant colonies (Lavoie and Campbell 2018). West-basin chicks' feathers were at the higher end of what has been reported at Great Lakes and inland sites, whereas east-basin chicks' feathers were on the lower end. For example, a survey of cormorant colonies across Canada found chick feather THg means varying from $\sim 1 \mu\text{g/g}$ at some Lake Ontario sites up to $\sim 8 \mu\text{g/g}$ at heavily Hg contaminated locations (Lavoie and Campbell

2018). Notably, that study sampled the first-grown primary feather (P1) in chicks, whereas my study used a later-grown P10 (and an R1 rectrix); because mercury deposition tends to decline with sequential feather growth as the internal Hg pool is depleted (Bottini et al. 2021), my feather values may slightly underestimate the maximum mercury concentrations that chicks accumulate in their earliest-grown feathers. Lavoie and Campbell (2018) sampled the east basin Mohawk Island colony for chick P1 feathers; the sample of 5 chicks had high variation (1800 ± 1300 ng/g) but was similar to my sampled mean mercury concentration of 1417.7 ± 274.5 ng/g ($n=15$) for P10 feathers.

4.4 Egg mercury and maternal transfer considerations

I found that both albumen and yolk mercury concentrations were not significantly different between Middle Sister Island (west) and Mohawk Island (east), indicating that breeding females at the two sites passed on similar mercury burdens to their eggs. However, similar egg mercury concentrations should not be taken as evidence that maternal body burdens were similar, because the fraction of a female's blood mercury that is deposited into eggs may decline as their own exposure rises (meta-analysis of 26 species: Ackerman et al. 2020). In other words, highly contaminated females transfer proportionally less of their mercury burden to each egg than lightly contaminated females, flattening the egg versus blood relationship at high exposure levels. This nuance means that west basin mothers could have higher blood mercury than east basin mothers while still producing eggs with similar mercury concentrations. This result is important because it rules out maternal deposition as an explanation for the large difference in chick mercury between basins. Therefore, chicks at both sites hatched with a similar initial mercury load acquired from the egg, and the striking divergence in their blood Hg by 3–5 weeks of age must have arisen post-hatch via differential dietary uptake.

My analysis of cormorant eggs showed that mercury was primarily MeHg in albumen and that albumen was significantly higher in mercury than yolk. Mercury in whole bird eggs is almost entirely in the methylated form (87–100% MeHg: Evers et al. 2003; Ackerman et al. 2013; Manceau et al. 2025), so measuring total Hg in eggs is a valid proxy for the bioavailable dose that the embryo/chick receives. Within the egg, however, mercury tends to partition disproportionately into the albumen compared to the yolk

(Magat and Sell 1979). I found that for yolk, percent MeHg was much lower at around 36–38%. Methylmercury has been found to be 67–86% of the total mercury present in yolk for several seabird species (Bond and Diamond 2009) and 32% for Emperor Penguins (Manceau et al. 2025). This disproportion of MeHg percentage between egg components is likely because MeHg binds strongly to proteins, and the albumen is protein rich (Magat and Sell 1979).

These egg mercury concentrations can also be compared to the literature toxicity thresholds of albumen from Ackerman et al. (2024) that I referenced previously; because mercury is mostly found in the albumen, yolk thresholds were not established. In that framework, albumen mercury concentrations of 0.15, 0.9, 2.0, and 4.6 $\mu\text{g/g}$ w.w. correspond to low, moderate, high, and severe injury (EC1, EC5, EC10, EC20), respectively. Assuming an albumen moisture content of approximately 90% for Double-crested Cormorant eggs (Dzialowski et al. 2009), my dry weight means correspond to approximately 0.13 $\mu\text{g/g}$ w.w. albumen in both basins, placing them just below the 0.15 $\mu\text{g/g}$ w.w. low injury benchmark. By contrast, west basin chicks had whole blood mercury spanning the low injury range and approaching the moderate injury benchmark (all individuals above the 0.3 $\mu\text{g/g}$ w.w. benchmark dose lower bound and the three highest reaching 0.50 $\mu\text{g/g}$ w.w., just below the 0.6 $\mu\text{g/g}$ w.w. moderate injury threshold; Ackerman et al. 2024). The difference in injury risk designations between eggs and chick blood emphasizes the importance of tissue choice when assessing contaminant effects in birds.

Though Lake Erie cormorant eggs are below the low-injury benchmark for albumen mercury concentrations, this alone provides limited insight into maternal risk. Because maternal transfer is non-linear and the proportion of a female's blood mercury transferred to eggs declines as her own exposure increases (Ackerman et al. 2020), similar egg mercury concentrations between basins should not be taken as evidence that maternal exposure was identical. Viewed alongside the large basin contrast in chick blood mercury, these results indicate that egg mercury is not a good proxy for maternal body burden, at least not at levels like those found across Lake Erie. Thus, adult female mercury levels need to be measured directly. Moreover, it would be valuable to clarify

how closely eggs reflect maternal body burden in Double-crested Cormorants at lower exposure levels where a relationship may be more apparent.

4.5 Chick trophic ecology and dietary niche

Because maternal transfer was similar between colonies, the striking inter-colony differences in chick mercury concentrations implicates dietary exposure as the primary driver of mercury spatial variation in Lake Erie cormorant chicks. Stable isotope analysis of chick tissues provided key insights into the trophic ecology of the two colonies, contributing to potential explanations of observed mercury patterns.

Two-baseline trophic position estimation using $\delta^{15}\text{N}$ showed that west basin chicks were feeding at higher trophic positions than east basin chicks (4.30 versus 3.78). This indicates that the west basin chicks were roughly half a trophic level higher than east basin chicks. In contrast, basin-specific relative benthic carbon use (α_R) of chicks did not differ markedly between the two sites. This suggests they had a similar mix of benthic versus pelagic energy sources in their diet. Thus, the primary dietary distinction was not in feeding habitat (nearshore benthic or pelagic offshore food webs) but rather in trophic position. However, it must be cautioned that, because trophic position estimates from $\delta^{15}\text{N}$ are baseline-dependent, the magnitude of this colony difference could be influenced by fine-scale spatial/seasonal baseline variability and by any mismatch between basin-level baselines and the prey web supporting chicks during the chick-rearing period.

Interpreting these colony-level patterns benefits from the basin-scale food-web framework reported by Heuvel et al. (2025). Using community-wide isotope metrics, they showed that the east basin of Lake Erie has a greater $\delta^{15}\text{N}$ range, whereas the west basin has shorter food chains but higher trophic diversity, consistent with high nutrient loading and productivity. Crucially, Heuvel et al. (2025) demonstrate that the same fish species often occupy different trophic positions by basin, with trophic positions generally lowest in the east and higher in the west. For example, walleye (west 4.2 vs east 3.6), yellow perch (3.7 vs 3.1), white perch (3.8 vs 3.2), and round goby (3.4 vs 3.0) all occupy higher trophic positions in the west basin versus east basin (Heuvel et al. 2025). They interpret this west to east decline in species-specific trophic position as consistent with the

eutrophication gradient, where the productive west basin supports more omnivorous behaviour due to the increased diversity of prey available.

I also analyzed regurgitated fish from each colony. A total of eight fish species were recovered and identified from opportunistically gathered regurgitated boli. In the east basin, I obtained pumpkinseed (*Lepomis gibbosus*), yellow perch (*Perca flavescens*), trout-perch (*Percopsis omiscomaycus*), round goby (*Neogobius melanostomus*), alewife (*Alosa pseudoharengus*), and young walleye (*Sander vitreus*). In the west basin, fewer species were sampled (partly due to fewer regurgitations available): round goby, smallmouth bass (*Micropterus dolomieu*), walleye, white perch (*Morone americana*), and yellow perch. With respect to prey composition, my opportunistic regurgitation sample sizes were too limited to demonstrate colony-level dominance of particular prey; therefore, I interpret provisioning patterns primarily through published diet studies and use my samples as qualitative context.

Double-crested Cormorant dietary studies demonstrate clear contrasts between the two Lake Erie basins. Stomach analysis of 302 adults collected in 1997 near West Sister Island (~15 km away from Middle Sister Island) showed that young-of-year gizzard shad (48 % of prey biomass), freshwater drum (33 %), and emerald shiner (9 %) dominated the diet, with sport fish each contributing < 4 % (Bur et al. 1999). In the east basin, round goby accounts for 70% of regurgitated prey items at the Mohawk Island colony (2009–2010; King et al. 2017) and as much as 85% of stomach biomass in Upper Niagara River birds (50 km away from Mohawk Island; 2004–2007; Coleman et al. 2012). The remaining species (with >1% contribution) found at Mohawk Island included Alewife (2%), cyprinid shiners (likely 13.9%), yellow perch (4.7%), smallmouth bass (2.7%), and common mudpuppy (2.6%). Thus, established literature predicts a shad- and drum-based diet, with trophic positions of 3.3 and 3.8, respectively, at Middle Sister Island (west basin), and a goby-based diet (trophic position 3.0) at Mohawk Island (east basin; Heuvel et al. 2025). These dietary contrasts align with a higher chick trophic position in the west basin, but their interpretation should be tempered by the age of the diet studies and the knowledge that cormorant diets can shift rapidly with prey availability changes.

Because colonies had similar mercury burdens at hatch, it can be concluded that chicks in the west basin were provisioned with prey carrying higher mercury concentrations than those fed to chicks in the east basin. This may reflect biomagnification through feeding at a higher trophic position and/or differences in local contamination. The opportunistic feeding ecology of Double-crested Cormorants means that diet composition can vary markedly among colonies, leading to corresponding variation in contaminant exposure (King et al. 2017). My results are consistent with this pattern: although both colonies are within Lake Erie, regional differences in food-web structure appear sufficient to produce higher chick mercury burdens in the west, where provisioning fish are more predatory. However, disentangling this dietary effect from higher baseline contamination in the west basin linked to historical industrial inputs remains challenging. Understanding these interacting factors is essential, as spatial variation in diet can mask or exaggerate the true effects of contamination (King et al. 2017).

4.6 Reliability of trophic position estimation

Assessing the robustness of these basin-level trophic differences requires considering how sensitive $\delta^{15}\text{N}$ -based trophic position estimates are to baseline choice and temporal mismatch (Post 2002; Stephens et al. 2023). The estimated chick trophic positions are consistent with basin-scale food-web patterns reported for Lake Erie fishes, where trophic position for most fish species showed a modest decline from west to east when estimated using basin-specific baselines (Heuvel et al. 2025). Taken together, my chick TP results and the fish-community analysis support the hypothesis that food-web structure differs among basins and that predators in the west basin may integrate slightly longer or higher-trophic pathways than predators in the east basin (Post 2002; Heuvel et al. 2025). At the same time, however, $\delta^{15}\text{N}$ -derived trophic position estimates remain baseline-dependent, and uncertainty can persist even when using basin-specific baselines (Post 2002; Stephens et al. 2023).

In my study, trophic position was estimated using two baseline groups (dreissenids representing the pelagic pathway and oligochaetes representing the benthic pathway), and baseline means were assigned basin-specifically to reduce spatial bias (Post 2002; Heuvel et al. 2025; this study). However, even primary consumers such as dreissenid mussels can

show strong spatial and seasonal variability in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and this variability can meaningfully shift consumer trophic position estimates depending on which baseline location/month is used (Guzzo et al. 2011). In western Lake Erie specifically, Guzzo et al. (2011) found that month explained a large fraction (>50%) of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ variability in zebra mussels and reported that fish trophic position estimates could vary by up to ~ 0.7 trophic levels depending on the zebra mussel baseline chosen (Guzzo et al. 2011). This matters for interpreting my basin comparison because the baseline data I relied on (Heuvel et al. 2025) were collected in a different year and across a broader seasonal window (May–November 2019) than my chick sampling (mid-June; 2022–2024), so temporal mismatch remains a plausible contributor to apparent TP differences (Guzzo et al. 2011; Heuvel et al. 2025).

To relate trophic position differences to mercury, it helps to separate trophic magnification slope (TMS) from trophic magnification factor (TMF), two common ways to summarize biomagnification in aquatic food webs (Lavoie et al. 2013). TMS is the slope of the relationship between $\log_{10}(\text{Hg})$ (often MeHg) and $\delta^{15}\text{N}$ across trophic levels (Lavoie et al. 2013). In their worldwide meta-analysis, the mean MeHg TMS was 0.24 ± 0.08 (range 0.08–0.53), showing that the strength of biomagnification varies among food webs (Lavoie et al. 2013). TMF is a ‘per trophic level’ multiplier (TMF = 6 means ~ 6 times higher Hg per trophic level; Lavoie et al. 2013). The TMF can be calculated from TMS using the typical $\delta^{15}\text{N}$ enrichment per trophic level ($\sim 3.4\text{‰}$; Post 2002; Lavoie et al. 2013). Using the global mean MeHg TMS (0.24) gives a TMF of ~ 6.55 , so half a trophic-position difference (0.5) would predict a MeHg difference of ~ 2.56 (Lavoie et al. 2013). The observed ~ 3 -fold west–east difference in chick blood mercury is therefore broadly consistent with (but does not prove) a trophic-position shift of roughly half a trophic level (Post 2002; Lavoie et al. 2013).

Importantly, my results also show that evidence for within-basin biomagnification differs by basin, which cautions against interpreting the between-basin mercury contrast as purely trophic. Within the east basin, trophic position was strongly and positively correlated with both THg and MeHg in chick red blood cells, consistent with biomagnification within that basin. In contrast, no relationship was detected between

trophic position and either THg or MeHg in west-basin chicks, indicating that trophic position did not explain among-individual mercury variability in the west. One interpretation is that west-basin chick mercury is more strongly influenced by spatial heterogeneity in baseline mercury bioavailability and/or prey-specific mercury differences at similar trophic position, which can obscure a simple Hg–TP relationship. Therefore, trophic structure and Hg hotspots may both contribute to the observed west–east mercury contrast and cannot be fully disentangled here.

4.7 Chick dietary mercury

To directly assess dietary mercury exposure, I analyzed Hg concentrations in muscle from regurgitated fish. Although sample sizes were limited, the data provide a glimpse into potential differences in prey mercury between the west and east basins.

Among east-basin prey, pumpkinseed sunfish stood out with exceedingly high mercury relative to other species: the mean for eight individuals was 1,827 ng/g d.w. THg, which is the highest of any prey type I measured. This is quite elevated for a small sunfish (most likely reflecting localized Hg enrichment in the benthic food chain of that area, or the age of the sampled fish). By contrast, round gobies from the east had much lower THg (113 ng/g d.w.), and alewife and trout-perch were similarly low (~135–150 ng/g d.w.). The young walleye (~23.5 cm length) from the east had intermediate THg (mean ~719 ng/g d.w.), consistent with being higher trophic predators but still relatively small individuals.

The east-basin diet seemed to be composed largely of low-mercury prey, with the exception of the pumpkinseeds which, if eaten often, could be a large contributor to chick mercury ingestion. However, nitrogen stable isotope values indicated the chicks' overall diet in the east did not strongly reflect pumpkinseed consumption (pumpkinseed $\delta^{15}\text{N}$ was too low for high abundance in chick diet). This suggests that pumpkinseeds were likely an infrequent or opportunistic component of the diet. This is further corroborated by the absence of pumpkinseed in Lake Erie cormorant dietary studies (Bur et al. 1999; Coleman et al. 2012; King et al. 2017). East basin chicks likely ate the lower mercury gobies, perch, alewife or other prey fish with similar mercury burdens the majority of the time.

In the west basin, the limited fish samples prevent much interpretation, but some intriguing observations can be made. A single round goby contained 967 ng/g d.w. of total mercury, which was several times higher than the mean of east basin gobies (112.7 ng/g d.w.). While based on one individual, this hints at a potential mercury hotspot in the west basin if it is representative of other round goby mercury burdens. Western Lake Erie has legacy mercury from industrial inputs, and round goby there might feed on higher mercury benthic invertebrates such as dreissenid mussels (Hogan et al. 2007). The three walleye from the west averaged 428 ng/g d.w., lower than the east walleye average, but notably the west walleye were smaller (fork length mean 16.9 cm west vs 23.5 cm east). Mercury in fish generally increases with length and age of fish (Zrnčić et al. 2013), so this does not necessarily indicate cleaner conditions in the west, it may simply reflect younger or smaller fish. While these data hint at differences, with only 1–3 individuals for most west-basin species, and an unbalanced species list, I cannot conclude anything about west versus east basin fish mercury burdens through my regurgitate data alone.

Despite Lake Erie's long history of contaminant monitoring, peer-reviewed studies that explicitly compare fish mercury burdens between the western and eastern basins are scarce. The most detailed lake-wide analysis is a 35-year data synthesis by Azim et al. (2011), which applied Bayesian models to Ontario Ministry of the Environment and Environment Canada fillet records. Average total mercury in walleye filets from the western basin (0.205 $\mu\text{g/g}$ w.w.) was consistently lower than in the eastern basin (0.227 $\mu\text{g/g}$ w.w.) despite higher sediment mercury concentrations in the west basin (Azim et al. 2011; Lepak et al. 2015). The underlying drivers of this somewhat counterintuitive pattern remain unclear; they may reflect differences in mercury contamination sources, variations in food web length and structure, biotic tissue turnover rates, local biogeochemical conditions, and species-specific home ranges (Azim et al. 2011). Although the western basin has historically been more polluted, its shallow depth, high productivity, and warmer temperatures can accelerate walleye growth and thus dilute tissue contaminant levels. Walleye also have extensive home ranges, spanning thousands of square kilometers (Bihun et al. 2024). Therefore, they are not a good indicator of very local site contamination. Azim et al. (2011) also looked at yellow perch, which are more localized, and found the opposite trend to walleye: contaminant

concentration was higher in fish collected from the west basin (0.136 $\mu\text{g/g ww}$) than in fish from the east basin (0.108 $\mu\text{g/g ww}$). More comprehensive sampling is needed to clarify west–east basin differences in fish mercury burdens.

5 Conclusion

My study demonstrates that post-hatch diet is the primary driver of spatial variation in mercury exposure for Double-crested Cormorant chicks in Lake Erie. Red blood cell mercury concentrations in 3- to 5-week-old chicks were roughly three-fold higher at Middle Sister Island (west basin) than at Mohawk Island (east basin), yet egg mercury levels were statistically indistinguishable between colonies. Apparent equal levels of maternal transfer therefore rule out pre-hatch loading as the cause of the disparity and implicates differences in chick diet. Stable isotope data suggest that west basin chicks occupied a higher trophic position. Prey mercury burdens in the west versus east basin are still unclear, with some species showing opposite patterns. Despite greater sediment and water column mercury concentrations in the west basin (Dove et al. 2012; Lepak et al. 2015), current literature is not comprehensive enough to suggest a clear basin-specific pattern in mercury concentrations in Lake Erie fish. Thus, geographically uneven trophic feeding within the lake may translate rapidly into unequal contaminant burdens at an early developmental stage, though the relative contributions of local contamination and food-web structure remain unresolved; further fine-scale mercury and stable isotope sampling is needed to distinguish between them. It remains possible that trophic position estimates are inflated by eutrophication-driven enrichment of $\delta^{15}\text{N}$ that the baseline correction does not fully resolve — in which case equal trophic positions would imply that west basin chicks are being provisioned from a localized hotspot of bioavailable mercury contamination not yet captured in the literature.

Overall, mercury risk is not uniform within Lake Erie for Double-crested Cormorant chicks: localized food-web pathways deliver elevated methylmercury to these upper trophic predators during a sensitive developmental period. Because chick tissues integrate exposure over weeks and respond quickly to dietary conditions, Double-crested Cormorant colonies can act as practical indicators for identifying persistent mercury

hotspots. Targeted sampling of prey fish and isotopic baselines across basins will be essential to separate the roles of food-web structure and local contamination and to pinpoint the sources and pathways driving higher exposure in western Lake Erie.

5.1 Study limitations

Field conditions on Middle Sister Island in the west basin constrained sampling. A mass mortality event affecting multiple waterbird species, including Double-crested Cormorants, limited the number and balance of biological samples collected. This lowered statistical power and only included living individuals and their gut contents that were accessible under those conditions. In particular, prey-fish sample size was small and were unlikely to capture the diversity or proportional availability of chick diets, limiting inference about links between specific prey and observed variation in chick mercury. In addition, more than a single field visit is needed to capture the variety of cormorant diets as they have been found to vary temporally during the breeding season (Neuman et al. 1997).

Exact chick ages could not be recorded at the nest. Body mass was used as a proxy for age; however, mass exhibits short-term variability (post-feeding) and individual condition differences. To partially compensate for this uncertainty, feathers can act as a temporal anchor: feather mercury reflects the discrete interval of feather growth, providing a standardized developmental time point across individuals even when exact ages are unknown. Feather mercury showed the same spatial differences in mercury concentrations, indicating age differences are very unlikely to be the cause.

As discussed in Section 4.6, $\delta^{15}\text{N}$ -based trophic position estimates are sensitive to baseline selection and timing. Because baseline values were not co-sampled within the same places and weeks as chick tissues, the absolute TP estimates and the precise magnitude of the west–east TP difference should not be interpreted as definitive. Consequently, while the observed west–east Hg contrast is consistent with higher trophic feeding, this study cannot definitively separate the role of biomagnification from spatial heterogeneity in MeHg bioavailability within the west basin.

Finally, dietary analysis was limited to $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. With many potential prey sources and only two bulk isotope tracers, diet mixing is under-determined; quantitative source apportionment was therefore not attempted. Compound-specific amino-acid isotope analyses or additional tracers would improve dietary attribution and strengthen interpretation of spatial patterns.

5.2 Future directions

Future research should include broader and balanced sampling of cormorant prey species and their contaminant levels across both basins to clarify dietary mercury sources, ideally incorporating seasonal and annual variability to better capture temporal patterns and their implications. Prey contaminant sampling should be paired with co-occurring isotopic baseline sampling within the colony's foraging footprint (benthic and pelagic primary consumers), allowing trophic position to be estimated using the relevant isoscape. This would help distinguish whether west–east differences in chick mercury burdens primarily reflect biomagnification via diet/trophic pathways or spatial differences in baseline methylmercury bioavailability. Monitoring of chick cohorts from hatch through fledging and biochemical assessments focusing on oxidative stress markers, immune function parameters, and neurobehavioral development could also provide insights into potential sub-lethal impacts on cormorant health. Given the elevated mercury exposure at critical developmental stages, cormorant chicks in the west basin may face heightened risks of adverse health and fitness outcomes, emphasizing the need for targeted monitoring efforts in this population.

The mass-mortality event recorded at the west-basin colony occurred amid a continental panzootic of highly pathogenic avian influenza (HPAI H5N1) that caused large die-offs in many avian species, particularly colonial waterbirds, since 2021 (Tian et al. 2023). While there is no necropsy or virology from this site, HPAI-linked die-offs in Great Lakes colonial birds and confirmed H5N1 exposure in cormorants elsewhere underscore disease pressure during my study period. Any discussion of mechanism must be explicitly tentative, but a plausible hypothesis is that sublethal contaminant burdens could reduce resilience to infectious disease by subtly depressing immune function or increasing physiological stress, thereby lowering survival under epidemic conditions.

Field and experimental work show that MeHg can reduce reproductive output and alter behavior, and multiple studies report immunomodulatory effects, as well as oxidative-stress gene responses in cormorants as mercury rises (Lewis et al. 2012; Gibson et al. 2014). In contrast to the west basin, the east basin (where chicks have lower mercury burdens) had no mass reported die-offs. These lines of evidence do not establish causation for this event, but they provide compelling motivation for an integrated disease-contaminant framework for colonial waterbirds in the Laurentian Great Lakes region, and elsewhere.

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Appendices

Appendix A: Chick Stable Isotope Values

Table A1. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope values of Double-crested Cormorant chick red blood cell samples at two Lake Erie sites: Middle Sister Island (MS; west basin) and Mohawk Island (MI; east basin).

| Island | Date | $\delta^{13}\text{C}$ | $\delta^{15}\text{N}$ |
|--------|------------|-----------------------|-----------------------|
| MI | June 28/22 | -23.66 | 16.76 |
| MI | June 28/22 | -23.55 | 15.97 |
| MI | June 28/22 | -23.32 | 16.02 |
| MI | June 28/22 | -23.37 | 15.90 |
| MI | June 28/22 | -23.30 | 15.95 |
| MI | June 28/22 | -23.34 | 16.13 |
| MI | June 28/22 | -22.43 | 16.11 |
| MI | June 28/22 | -23.48 | 16.15 |
| MI | June 28/22 | -23.62 | 15.81 |
| MI | June 28/22 | -23.55 | 16.02 |
| MI | June 28/22 | -23.38 | 16.00 |
| MI | June 28/22 | -23.54 | 16.13 |
| MI | June 29/23 | -23.38 | 16.92 |
| MI | June 29/23 | -22.84 | 16.85 |
| MI | June 29/23 | -22.62 | 16.85 |
| MI | June 29/23 | -22.84 | 16.91 |
| MI | June 29/23 | -24.03 | 16.82 |
| MI | June 29/23 | -23.12 | 16.73 |

| | | | |
|----|------------|--------|-------|
| MI | June 29/23 | -22.78 | 16.90 |
| MI | June 29/23 | -23.38 | 16.92 |
| MI | June 29/23 | -23.42 | 16.61 |
| MI | June 29/23 | -23.32 | 16.49 |
| MI | June 29/23 | -23.69 | 16.68 |
| MI | June 29/23 | -23.66 | 16.76 |
| MI | June 16/24 | -24.03 | 16.82 |
| MI | June 16/24 | -23.32 | 16.49 |
| MI | June 16/24 | -22.62 | 16.85 |
| MI | June 16/24 | -23.06 | 16.83 |
| MI | June 16/24 | -25.07 | 15.39 |
| MI | June 16/24 | -23.69 | 16.68 |
| MI | June 16/24 | -23.12 | 16.73 |
| MI | June 16/24 | -22.78 | 16.90 |
| MI | June 16/24 | -23.42 | 16.61 |
| MI | June 16/24 | -22.84 | 16.91 |
| MI | June 16/24 | -23.31 | 16.60 |
| MI | June 16/24 | -23.89 | 16.42 |
| MI | June 16/24 | -23.54 | 16.50 |
| MI | June 16/24 | -24.32 | 16.76 |
| MI | June 16/24 | -23.87 | 16.23 |
| MS | July 3/22 | -21.46 | 16.43 |
| MS | July 3/22 | -21.81 | 16.07 |
| MS | July 3/22 | -21.67 | 15.32 |
| MS | July 3/22 | -22.17 | 14.96 |

| | | | |
|----|------------|--------|-------|
| MS | July 3/22 | -21.44 | 15.83 |
| MS | July 3/22 | -21.21 | 16.34 |
| MS | July 3/22 | -20.52 | 14.98 |
| MS | July 3/22 | -21.94 | 15.22 |
| MS | June 15/24 | -21.24 | 15.73 |
| MS | June 15/24 | -20.89 | 15.56 |
| MS | June 15/24 | -21.24 | 15.33 |
| MS | June 15/24 | -21.41 | 14.22 |
| MS | June 15/24 | -21.65 | 14.25 |
| MS | June 15/24 | -21.5 | 14.50 |
| MS | June 15/24 | -21.29 | 16.27 |

Mean (SD) for MS (n = 15): $\delta^{13}\text{C} = -21.43 (0.41)$, $\delta^{15}\text{N} = 15.40 (0.73)$

Mean (SD) for MI (n = 39): $\delta^{13}\text{C} = -23.40 (0.50)$, $\delta^{15}\text{N} = 16.49 (0.40)$

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