

**Effect of lampricide exposure on olfaction and
stress physiology in juvenile lake sturgeon
(*Acipenser fulvescens*)**

by

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for the M.Sc. in Biology

Department of Biology

Algoma University

A handwritten signature in black ink that reads "C. Maden".A handwritten signature in blue ink that appears to be "WAD".

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Declaration of Authorship

I hereby declare that this thesis incorporates material resulting from joint research as follows:

The key ideas, primary contributions, experimental design, data analysis, interpretation, and writing of each chapter were undertaken by myself, Michelle Monteiro, under the supervision of Dr. Christine Madliger and Dr. William Dew.

In Chapter 1 (General Introduction) and Chapter 3 (General Discussion), I present unpublished material written entirely by myself, under the supervision of Dr. Christine Madliger and Dr. William Dew.

I have prepared Chapter 2 as a manuscript with intention for publication. I completed the manuscript, including study design, data analysis, interpretation, and writing, with input from Dr. Christine Madliger, Dr. William Dew, and Dr. Trevor Pitcher who will be included as co-authors on the publication. Olivia Galloway and Stuart Ness assisted in collecting blood samples and conducting glucose and whole-blood analyses for a subset of samples, and will also be included as co-authors on the publication arising from Chapter 2.

I certify that I have properly acknowledged the contributions of all other researchers to my thesis and have obtained written permission from each co-author to include the above materials in my thesis.

I certify that, with the above qualifications, this thesis, and the research to which it refers, is the product of my own work.

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Abstract

Freshwater ecosystems are facing increasing pressures from invasive species, habitat alteration, and chemical pollutants. In the Laurentian Great Lakes, lampricides have been used for decades to suppress invasive sea lamprey, and the sea lamprey program is widely credited as a critical component of the recovery and maintenance of native fisheries. However, the application of these chemicals in nursery streams also creates potential risk for non-target species of conservation concern, such as lake sturgeon (*Acipenser fulvescens*), whose early life stages often overlap spatially and temporally with treatment zones. While young-of-the-year (YOY) sturgeon have been shown to exhibit functional impairments following lampricide exposure, the vulnerability of older juveniles remains unclear. To address this gap, I investigated whether short-term exposure to TFM or a TFM with 1% niclosamide mixture affects olfactory sensitivity or stress physiology in yearling (age 1+) lake sturgeon. I exposed fish for either 30 minutes or 12 hours and evaluated responses immediately after exposure and following a six-day recovery period. I measured plasma cortisol and glucose concentrations to evaluate baseline endocrine function, responsiveness to handling stress, and post exposure patterns over time. I used electro-olfactography to assess activation of microvillous and ciliated olfactory sensory neurons using L-alanine and taurocholic acid as stimuli. I found no treatment-related effects on baseline cortisol, cortisol responsiveness, circulating glucose, or stress-induced glucose, indicating that endocrine and energetic function remained intact under the exposure conditions used in this study. I also observed no reductions in EOG amplitudes, suggesting that peripheral olfactory sensitivity was not impaired. In contrast to YOY sturgeon, my results indicate that yearling lake sturgeon may have greater physiological tolerance to lampricide exposure, particularly under

moderate alkalinity and near-neutral pH conditions. Overall, my research adds to a growing body of literature that collectively demonstrates that non-target vulnerability to lampricides is strongly influenced by life stage, physiological development, and environmental context, and contributes evidence that can improve life-stage-specific risk assessment and conservation planning for lake sturgeon.

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Land Acknowledgement

My research was conducted in Robinson-Huron Treaty territory, on the traditional lands of the Anishnaabeg, and within the homelands of the Garden River and Batchewana First Nations and Métis People. My data collection also took place on the traditional territory of the Three Fires Confederacy of First Nations, which includes the Ojibwa, the Odawa, and the Potawatomi. I acknowledge the deep history and ongoing contributions of these communities to this land and our collective responsibility to respect and uphold their rights, histories, and knowledge systems.

Declaration of Environmental Impact

I acknowledge that my thesis project involved activities that had an environmental impact, and I outline here some of the steps taken to lessen it. The research required travel between Sault Ste. Marie and LaSalle, Ontario, as well as regular trips between our accommodations and the research facility. As a team, we carpoled whenever possible, both for long-distance travel and for daily commutes, which helped reduce fuel use and emissions. We also stayed in shared accommodations to limit our overall footprint.

The work at the Freshwater Restoration Ecology Centre and later at Algoma University required the use of both reusable and single-use materials. We reused equipment and containers wherever possible, cleaning and storing them for future work. However, certain single-use plastics were unavoidable, especially for blood sampling and processing, where sterility and sample integrity were important. All materials were disposed of according to the safety and environmental guidelines in place at the institution.

There is still room to reduce the overall impact that scientific research has on the environment, and I will continue to try to make responsible choices in my future work.

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Ethical Compliance Statement

This research was conducted in compliance with ethical animal use standards. Institutional animal care permits were granted by Algoma University (AUP 2024-CM-BD-Sturgeon-01) and the University of Windsor (AUP-24-08).

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Chapter 1 - General Introduction

Freshwater ecosystems

Freshwater ecosystems represent some of the most productive and biologically diverse habitats on Earth despite covering less than one percent of the planet's surface area (Dudgeon et al. 2006; Strayer and Dudgeon 2010). They support over ten percent of all known species, including approximately thirty percent of vertebrates and forty percent of global fish diversity (Dudgeon et al. 2006; Strayer and Dudgeon 2010). Their high biodiversity stems from habitat diversity and ecological isolation, which promoted rapid speciation relative to marine systems (Miller 2021). Freshwater systems also provide irreplaceable ecosystem services such as potable water, inland fisheries, nutrient cycling, and flood regulation, among others, that sustain both ecological and human well-being (Apostolaki et al. 2020; Lynch et al. 2023). Freshwater systems are increasingly constrained by multiple interacting stressors including flow alteration, pollution, habitat fragmentation, and climate change (Dudgeon et al. 2006; Strayer and Dudgeon 2010; Reid et al. 2018). Dams and impoundments homogenize hydrologic regimes, reduce sediment transport, and disrupt connectivity essential for fish migration (Kondolf 1997; Poff et al. 1997; Nilsson et al. 2005). Agricultural and industrial runoff drives eutrophication and chemical contamination, degrading water quality and facilitating invasion by tolerant species (Carpenter et al. 2011; Alexander et al. 2017). Chemical stressors can alter metabolism and sensory function in non-target fishes (Birceanu et al. 2014; Sakamoto et al. 2016). As a result of these combined anthropogenic stressors, freshwater ecosystems are declining faster than their marine and terrestrial counterparts; global freshwater vertebrate

populations have declined by over 80% since 1970 and one-quarter of taxa are now threatened with extinction (Reid et al. 2018; Abell and Harrison 2020; Sayer et al. 2025).

Multiple stressors in freshwater systems can result in additive and more than additive effects, amplifying physiological stress and ecological decline (Folt et al. 1999; Jackson et al. 2016). For example, manganese exposure combined with elevated temperature has been associated with reduced aerobic capacity in juvenile Arctic charr (*Salvelinus alpinus*), a pattern the authors suggest may reflect temperature-dependent effects on mitochondrial function (Garnier et al. 2025). Sensory pollutants such as artificial light further disturb circadian rhythms, predator-prey interactions, and reproductive behaviour (Dominoni et al. 2016; Moyses et al. 2023). Individually, TFM and niclosamide act as chemical stressors, but their combined effects become synergistic, producing greater toxicity (Boogaard et al. 2007; Hepditch et al. 2021). These compounded pressures weaken community stability and alter population structure, emphasizing the need to evaluate a wide array of stressors to understand how aquatic ecosystems are affected.

Conservation status of North American freshwater fishes

North American freshwater fishes are among the most imperiled vertebrate groups globally. Nearly thirty-nine percent of described species are considered vulnerable, threatened, endangered, or extinct, which represents a 92% increase since 1989 (Jelks et al. 2008). Extinction rates are estimated to be 800-900 times higher than background levels, with up to 86 additional species projected to disappear by 2050 (Burkhead 2012). Habitat modification, invasive species, pollution, and overexploitation remain primary drivers (Dudgeon et al. 2006;

Reid et al. 2018). Recovery progress remains limited; only approximately six percent of imperiled taxa have improved in status, while most continue to decline (Jelks et al. 2008). Without large-scale conservation intervention, biodiversity loss in North American freshwater systems is expected to accelerate (Burkhead 2012; Tickner et al. 2020). Understanding these broad patterns of decline also requires attention to the targeted management actions used in freshwater ecosystems, including chemical control programs designed to suppress invasive species.

History of lampricide use in sea lamprey control

The parasitic sea lamprey (*Petromyzon marinus*) invaded the Great Lakes in the early 1900s following the construction of the Welland Canal, which bypassed Niagara Falls and allowed the species to access the upper Great Lakes (Lawrie 1970; Smith and Tibbles 1980). By the mid-1940s, sea lamprey predation had devastated native fish populations, including lake trout (*Salvelinus namaycush*), and was described as an “international catastrophe for the fisheries” of the Great Lakes (Fetterolf Jr. 1980; Smith and Tibbles 1980; Figure 1.1). In response to these losses, the Great Lakes Fishery Commission (GLFC) was established in 1955 under the Convention on Great Lakes Fisheries, a binational agreement between Canada and the United States that coordinated fishery management and initiated a comprehensive program to control sea lamprey populations (Fetterolf Jr. 1980; Gaden 2007). The Commission’s formation marked a pivotal step toward managing the Great Lakes as a unified ecological and economic system (Eshenroder 1987; Gaden 2007). Since the early 1960s, the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) has been the cornerstone of the GLFC’s sea lamprey control program (Smith

and Tibbles 1980; Bills et al. 2003; Boogaard et al. 2003; Figure 1.1). Treatments are applied to larval nursery streams every two to four years, eliminating several lamprey cohorts within a single treatment cycle (Bills et al. 2003; McDonald and Kolar 2007). TFM disrupts oxidative metabolism by uncoupling mitochondrial respiration, leading to decreased ATP production and death in sea lamprey larvae due to energy depletion (Niblett and Ballantyne 1976; Birceanu et al. 2009). To ensure effective control, TFM is administered at approximately 1.2-1.5 times the minimum lethal concentration (MLC), a margin that accounts for downstream dilution and dispersal (O'Connor et al. 2017).

The operational concentration required during a treatment is determined largely by site-specific water chemistry, especially pH and alkalinity, because these factors govern the proportion of TFM present in its protonated and unprotonated forms (Bills et al. 2003; Wilkie et al. 2019). TFM is a weak acid, and at lower pH a greater fraction of TFM exists in its un-ionized (protonated) form, which is more readily absorbed across gill membranes and thus more toxic to sea lamprey (Wilkie et al. 2019; Wilkie et al. 2021; Figure 1.2). Conversely, higher pH conditions favour the ionized (unprotonated) form, reducing uptake and requiring higher treatment concentrations (Wilkie et al. 2019; Wilkie et al. 2021; Figure 1.2). Alkalinity, which represents the buffering capacity of water, also influences TFM toxicity (Cole 1988; Hepditch et al. 2019). Although it does not alter TFM speciation directly, higher alkalinity reduces gill microenvironment acidification caused by CO₂ and metabolic acid excretion, thereby decreasing local TFM bioavailability at the gills (Wilkie et al. 2021). As a result, sea lamprey and non-target fishes exhibit greater sensitivity to TFM in poorly buffered, low-alkalinity waters (Hepditch et al. 2019; Wilkie et al. 2021). Young-of-the-year (YOY) lake sturgeon exhibit two- to

three-fold higher TFM uptake than older juveniles, likely due to their higher mass-specific metabolic rates (Hepditch et al. 2019). These effects are intensified in low-alkalinity conditions, where greater protonation enhances TFM bioavailability and increases the risk of non-target toxicity during early life stages (Hepditch et al. 2019).

TFM is frequently combined with niclosamide (2',5-dichloro-4'-nitrosalicylanilide), which significantly enhances its effectiveness, allowing lower concentrations of TFM to achieve the same level of sea lamprey control (Boogaard et al. 2007). Niclosamide functions as a more potent uncoupler of mitochondrial oxidative phosphorylation than TFM, leading to increased toxicity in sea lamprey (Borowiec et al. 2022). Similar to TFM, niclosamide exists in both protonated and unprotonated forms, with the protonated form being more toxic to sea lamprey (Wilkie et al. 2019; Figure 1.2). The addition of granular niclosamide is particularly valuable in lentic or deepwater environments where dilution would render TFM ineffective (Sullivan et al. 2021).

Sensitivity to lampricide

The introduction and refinement of lampricide treatments have reduced sea lamprey abundance across the Great Lakes by roughly 90% from historical peak levels (Pearce et al. 1980; Heinrich et al. 2003; Lavis et al. 2003; Siefkes 2017). This success is largely attributed to the sea lamprey's limited ability to detoxify TFM (Lech 1974; Lech and Statham 1975; Clarke et al. 1991; Kane et al. 1994; Lawrence et al. 2022). Specifically, TFM and niclosamide disrupt essential physiological processes in fish, ultimately leading to mortality (Wilkie et al. 2019; Borowiec et al. 2022). TFM primarily interferes with cellular respiration by uncoupling oxidative

phosphorylation in mitochondria, thereby disrupting ATP production, which is a critical source of cellular energy (Birceanu et al. 2011). In sea lamprey, the accumulation of TFM within tissues, coupled with less detoxification and excretion, amplifies this disruption and makes them particularly vulnerable (Clarke et al. 1991; Birceanu et al. 2009). Niclosamide acts through a similar but more potent mechanism, uncoupling mitochondrial oxidative phosphorylation at substantially lower concentrations than TFM (Borowiec et al. 2022). Laboratory studies in sea lamprey show that niclosamide is 40-60 times more potent than TFM at disrupting mitochondrial respiration, producing marked increases in State 4 respiration and sharp declines in respiratory control ratios (Borowiec et al. 2022). These effects reflect its superior ability to impair mitochondrial function and energy metabolism, making it highly toxic to sea lamprey, especially when used alongside TFM, as the combination enhances control efficacy while reducing TFM concentrations (Applegate et al. 1961; McDonald and Kolar 2007).

In most fishes, detoxification of lampricides occurs primarily through glucuronidation, a hepatic pathway in which UDP-glucuronosyltransferase (UGT) enzymes conjugate toxic compounds with glucuronic acid, increasing water solubility and promoting excretion (Lech and Statham 1975; Kane et al. 1994). Sea lamprey; however, exhibit greatly reduced glucuronidation capacity compared to other fishes (Lech 1973; Lech and Statham 1975). Limited phase II detoxification capacity in sea lamprey, particularly glucuronidation and sulfation, may prolong internal exposure to TFM and favor phase I nitroreductive pathways associated with bioactivation, rather than rapid elimination (Bussy et al. 2018a, b). The resulting biochemical disparity forms the basis of lampricide selectivity that allows effective sea lamprey suppression with minimal risk to many native species (Wilkie et al. 2019).

Most non-target fish species can tolerate TFM concentrations three to five times higher than those lethal to sea lamprey, although sensitivity varies widely among taxa (Applegate and King 1962; Bills et al. 2003; Wilkie et al. 2019). Despite its overall selectivity, evidence indicates that TFM can adversely affect some native fishes at concentrations used during standard field applications (Boogaard et al. 2003; Sakamoto et al. 2016; O'Connor et al. 2017). In rainbow trout (*Oncorhynchus mykiss*), exposure to sub-lethal TFM concentrations causes marked decreases in glycogen and ATP and increases in lactate within kidney and brain tissues, indicating severe metabolic disruption (Birceanu et al. 2009; Birceanu et al. 2014; Ionescu et al. 2021). TFM exposure has also been shown to transiently elevate plasma cortisol in trout, confirming activation of the hypothalamic-pituitary-interrenal (HPI) axis (Birceanu and Wilkie 2018).

TFM exposure also impaired olfactory function and related behaviours in lake sturgeon (*Acipenser fulvescens*) (Sakamoto et al. 2016). In young-of-the-year (YOY) lake sturgeon, electrophysiological recordings revealed reduced electro-olfactogram (EOG) amplitudes to a complex food cue, an amino acid (L-alanine) and a bile salt (taurocholic acid), suggesting inhibition of multiple olfactory sensory neuron types. Behavioural assays corroborated these results, showing diminished attraction to food-related odours and feeding rates following exposure (Sakamoto et al. 2016).

While many non-target species recover once exposure ceases, recovery rates vary among species, life stages, and environmental conditions. Rainbow trout, for instance, efficiently detoxify TFM via glucuronidation without long-term physiological impairment (Kane et al. 1994; Birceanu et al. 2014; LeClair and Wilkie 2014). However, for species with slow

maturation and low reproductive potential, such as lake sturgeon, even temporary disruptions in metabolism or sensory function could have lasting ecological consequences. There is therefore a need for continued assessment of lampricide exposure that incorporates metabolic, behavioural, and recovery endpoints to refine control strategies and minimize non-target risks.

Vulnerability of lake sturgeon (*Acipenser fulvescens*)

Lake sturgeon (*Acipenser fulvescens*) populations have declined drastically across North America due to overharvesting, habitat destruction, pollution, and the construction of dams that block access to spawning grounds (Ferguson and Duckworth 1997; LeBreton et al. 2004; Bruch et al. 2016; Figure 1.3; Figure 1.4). The Great Lakes-Upper St. Lawrence population of lake sturgeon is classified as 'Threatened' by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2017). As a long-lived, late-maturing species, they exhibit extremely slow population recovery, with models suggesting that full restoration can take more than a century following depletion (Vélez-Espino and Koops 2009; Pollock et al. 2015; Nelson et al. 2022). These life-history traits make them exceptionally sensitive to anthropogenic pressures, particularly those that elevate juvenile mortality. Juvenile and early life stages are further vulnerable to environmental stressors and chemical exposure (Tillitt et al. 2016; Yoon et al. 2019). Eggs and larvae experience high natural mortality, and early life stages are highly sensitive to brief environmental perturbations during incubation and drift, which can influence cohort survival (Bruch et al. 2006; Yoon et al. 2019). YOY sturgeon inhabit shallow nursery zones that often overlap with sea lamprey larval habitats, increasing their likelihood of lampricide exposure (Sakamoto et al. 2016; Pratt et al. 2021).

Laboratory and field studies have consistently shown that juvenile lake sturgeon are more sensitive to TFM than many other non-target fishes (Boogaard et al. 2003; O'Connor et al. 2017; Dobiesz et al. 2018; Hepditch et al. 2021; Ionescu et al. 2022). This pattern has been associated with small body size during early developmental stages, as well as elevated metabolic demand (Boogaard et al. 2003; Hepditch et al. 2019). Contrary to earlier assumptions, lake sturgeon are capable of detoxifying TFM through glucuronidation at levels comparable to rainbow trout, indicating that their heightened sensitivity is not due to a complete deficiency in this pathway, but likely arises from secondary factors such as reduced glycogen stores, size-dependent exposure, or metabolic constraints that limit their tolerance to TFM (Le Clair and Wilkie 2014). Beyond mortality, sublethal effects of TFM exposure present an additional conservation concern. Experimental work indicates that low-level lampricide exposure can impair olfactory function and disrupt stress physiology in sturgeon, potentially altering feeding or predator-avoidance behaviours essential for survival and recovery (Sakamoto et al. 2016). Combined with their potential vulnerability to parasitism by sea lamprey during early juvenile stages, these factors may collectively hinder population restoration efforts (Patrick et al. 2009; Hayes and Caroffino 2012; O'Connor et al. 2017; Dobiesz et al. 2018; Pratt et al. 2021).

Fish olfaction

The olfactory system in fish is essential for mediating critical behaviors such as locating food, avoiding predation, navigating migratory routes, recognizing mates and kin, and selecting spawning sites (Hara and Zielinski 2006; Derby and Sorensen 2008). Many species rely heavily

on chemical cues to interpret their environment and guide complex behaviours. For example, lake sturgeon are benthic feeders that depend on olfactory cues to detect prey buried in sediment, including aquatic insects, molluscs, and crustaceans (Harkness et al. 1961). Fish can also detect alarm cues released by conspecifics under threat, triggering evasive behaviors such as fleeing or sheltering (Wisenden 2000). Migratory fishes use olfactory cues to locate spawning habitat, but the mechanisms vary among taxa. Salmon home to natal streams using olfactory imprinting of stream-specific chemical signatures (Dittman and Quinn 1996), whereas sea lamprey rely on bile salt pheromones released by larvae to identify suitable spawning habitat rather than returning to natal streams (Waldman et al. 2008; Buchinger et al. 2014; Fissette et al. 2021). Overall, the fish olfactory system is remarkably sensitive, with odorant detection and response thresholds often in the nanomolar to picomolar range, and in some cases extending into the femtomolar range for biologically relevant pheromonal cues, as demonstrated by behavioral responses of sea lamprey to bile salts at concentrations as low as 10^{-14} M (Johnson et al. 2009; Døving and Kasumyan 2020). This acute sensitivity underlies complex behaviours such as long-distance navigation and homing to natal spawning grounds (Kleerekoper 1967; DeBose and Nevitt 2008). Because olfaction governs feeding, reproduction, and predator avoidance, disruption of this sensory pathway can have direct ecological and fitness consequences (Tierney et al. 2010).

Anatomy of the fish olfactory system

The olfactory system in fishes consists of paired olfactory organs located in nasal cavities on the snout (Olivares and Schmachtenberg 2019). In lake sturgeon, water enters through the incurrent naris, flows across a radial, rosette-like structure containing multiple sensory

lamellae, and exits via the excurrent naris, maintaining continuous odorant exposure (Garwood et al. 2019; Figure 1.5). This rosette arrangement slows water flow and enhances odorant–receptor contact, improving detection efficiency (Garwood et al. 2019). The olfactory epithelium (OE) is divided into sensory and non-sensory cells. The sensory cells consist of three principal types of olfactory sensory neurons (OSNs): ciliated OSNs, microvillous OSNs, and crypt OSNs (Zeiske et al. 2003; Hansen and Zielinski 2005; Olivares and Schmachtenberg 2019; Figure 1.5). This diversity of receptor types enables fish to process a wide range of chemical signals vital for survival and reproduction.

Electro-olfactogram (EOG)

An electro-olfactogram (EOG) is a quantitative, non-invasive technique used to measure the collective response of OSNs within the OE (Koce and Valentinčič 2000; Scott and Scott-Johnson 2002). The EOG records the summated generator potential produced when odorant molecules stimulate OSNs, detected as a transient negative voltage deflection at the surface of the OE (Scott and Scott-Johnson 2002; Figure 1.5). During recordings, a microelectrode is positioned between lamellae of the olfactory rosette to measure voltage changes, while a reference electrode establishes the baseline potential (Figure 1.5). The amplitude of the EOG response reflects the number of activated OSNs, whereas latency and recovery describe the response timing and return to baseline, respectively (Evans and Hara 1985; Nikonov et al. 2017). These parameters collectively provide a physiological measure of olfactory sensitivity and integrity. Common odorants such as L-alanine and taurocholic acid (TCA) are frequently used to test olfactory function because they activate distinct OSN classes, microvillous and ciliated neurons, respectively (Laframboise and Zielinski 2011; Dew et al. 2014). Since each OSN

class mediates specific odor-driven behaviours, EOG responses can indicate both the presence of sensory impairment and the potential behavioural consequences (Dew et al. 2014).

Effects of contaminants on fish olfaction

Disruption of olfactory function due to contaminants can significantly impair essential behaviours, with cascading effects on fish survival and population stability. Impaired detection of food cues can reduce foraging success, while loss of alarm or migratory cue detection increases predation risk and reproductive failure (Tierney et al. 2010; Dew et al. 2014). A wide range of contaminants including metals, organic contaminants, and surfactants have been shown to impair fish olfactory systems (Yue et al. 2025). Copper exposure primarily disrupts ciliated OSNs, while nickel selectively affects microvillous OSNs, demonstrating neuron-type-specific toxicity (Dew et al. 2014). Environmentally-relevant copper concentrations (3-58 µg/L) can reduce EOG amplitudes by up to 88%, and prolonged exposure can induce necrosis of OSNs (Hansen et al. 1999; Sandahl et al. 2006).

TFM also impairs olfactory sensitivity. Sakamoto et al. (2016) showed that exposure of YOY lake sturgeon to TFM significantly reduced EOG responses to L-alanine, taurocholic acid, and a cue derived from their food, indicating functional inhibition across multiple OSN types. Behavioural trials revealed that TFM-exposed sturgeon displayed weaker attraction to food scents and lower feeding rates compared to controls, linking physiological impairment to behavioural consequences. Although the duration of recovery from TFM exposure remains unknown, such sublethal effects have important implications for sturgeon conservation and habitat management. However, not all behavioural endpoints appear equally sensitive to TFM exposure; for example, Middaugh et al. (2014) reported no detectable effects of short-term

TFM exposure on growth or general avoidance behaviour in juvenile lake sturgeon, indicating that sublethal impacts may be endpoint- and context-dependent.

Stress physiology in fish

When a fish encounters a stressor such as handling, confinement, hypoxia, or exposure to some environmental contaminants, the hypothalamic-pituitary-interrenal (HPI) axis is activated (Wendelaar Bonga 1997; Barton 2002; Harper and Wolf 2009; García-Meilán et al. 2022). Activation begins when the hypothalamus releases corticotropin-releasing factor (CRF), stimulating the pituitary gland to secrete adrenocorticotrophic hormone (ACTH), which in turn signals interrenal cells, the functional equivalent of adrenal tissue in mammals, to synthesize and release cortisol into the bloodstream (Barton 2002; Portz et al. 2006). Following cortisol release, fish redirect energy use through multiple physiological pathways to maintain homeostasis (Mommsen et al. 1999). This transient rise in cortisol enhances gluconeogenesis and glycogenolysis, while temporarily downregulating energetically costly processes such as growth, reproduction, and immune activity (Mommsen et al. 1999). The secondary stress responses that follow include measurable changes in glucose and lactate, reflecting metabolic and hydromineral adjustments that support short-term survival (Barton 2002; Davis 2006). Overall, this endocrine cascade mobilizes energy reserves, promotes escape behaviour, and facilitates recovery following exposure to stress (Wendelaar Bonga 1997; Sapolsky et al. 2000). While acutely adaptive, prolonged activation of the HPI axis has been associated with suppressed growth, impaired immune function, reduced reproductive success, and increased disease susceptibility (Wedemeyer et al. 1990; Barton and Iwama 1991; Mommsen et al. 1999).

Disruption of the normal stress response may manifest as persistently elevated baseline cortisol, indicating a sustained stress state, or as a diminished capacity to mount an acute stress response, both of which can negatively affect growth, health and overall fitness (Sopinka et al. 2016).

In rainbow trout, exposure to TFM significantly elevates plasma cortisol levels, confirming activation of the HPI axis (Birceanu and Wilkie 2018). However, the duration and physiological consequences of this response remain poorly understood in lake sturgeon. Moreover, the combined use of TFM with niclosamide (TFM-N) has not been fully investigated for its sublethal impacts on non-target species. In sturgeon, already threatened by habitat degradation and slow recovery rates, the additional burden of chemically-induced or chronic stress could further hinder conservation efforts (Sakamoto et al. 2016; O'Connor et al. 2017; Dobiesz et al. 2018). Although sometimes complex in interpretation, cortisol remains the primary biomarker of stress in fish, provided that sampling occurs appropriately to avoid handling artifacts (Sopinka et al. 2016; Lawrence et al. 2018).

Links between the olfactory and stress pathway

In the context of fish survival, olfactory pathways and the stress axis are connected. Fish rely on olfaction to detect environmental cues, including those signaling predation risk, and such sensory input can activate the hypothalamic-pituitary-interrenal (HPI) axis, triggering the release of cortisol (Sanches et al. 2015; Barkhymer et al. 2019). Exposure to predator-related odours has been shown to elevate cortisol levels and induce behavioural changes such as heightened anti-predator responses, reduced feeding, and increased respiration in species like

zebrafish (*Danio rerio*) and Nile tilapia (*Oreochromis niloticus*) (Sanches et al. 2015; Barkhymer et al. 2019). Similarly, detection of predator-derived chemical cues elevates both cortisol and glucose concentrations in juvenile coho salmon (*Oncorhynchus kisutch*), reflecting activation of both endocrine and metabolic stress responses (Rehnberg and Schreck 1987). However, interspecific variation exists. For instance, while zebrafish show marked increases in whole-body cortisol under alarm cue exposure (Barkhymer et al. 2019), Nile tilapia and pumpkinseed (*Lepomis gibbosus*) exhibit minimal cortisol or glucose changes when exposed to predation odours (Miyai et al. 2016; Gallagher et al. 2019). These differences may arise from species-specific ecology, predator cue composition, and exposure duration (Sanches et al. 2015). Contaminants like TFM and niclosamide may simultaneously impair olfactory detection and activate stress pathways, amplifying physiological strain. Measuring both olfactory and endocrine responses therefore can provide a more comprehensive assessment of how lampricide exposure and other anthropogenic stressors influence fish health. This integrative approach is particularly valuable for the conservation of vulnerable species such as lake sturgeon, where cumulative sublethal effects could compromise recovery efforts (Sakamoto et al. 2016; Hepditch et al. 2019; Ionescu et al. 2021).

Knowledge gaps

Despite decades of successful sea lamprey control across the Great Lakes, the sub-lethal effects of lampricides on non-target fishes remain poorly understood under environmentally realistic exposure conditions (McDonald and Kolar 2007; Wilkie et al. 2019). Lake sturgeon, being a long-lived and late-maturing species of significant conservation concern, are particularly

vulnerable during early life stages that overlap with lampricide-treated nursery streams (Sakamoto et al. 2016; Pratt et al. 2021). While previous studies have identified acute metabolic and olfactory disturbances in YOY sturgeon following lampricide exposure, little is known about how yearling (i.e., age 1+) individuals respond. This developmental stage occurs after the larval drift period and coincides with increased movement capacity, broader habitat use, and continued ontogenetic changes in physiology and energetics relative to earlier life stages (Dettlaff et al. 1993; Peterson et al. 2007). Key knowledge gaps persist in understanding how lampricides affect olfactory and endocrine function in yearling lake sturgeon. Most existing research has examined these endpoints in isolation (Sakamoto et al. 2016; Ionescu et al. 2021, 2022), overlooking the potential interactions between sensory disruption and physiological stress responses. Evaluating both olfactory sensitivity and endocrine indicators such as cortisol and glucose across multiple recovery periods can also provide a more integrated understanding of sub-lethal effects. Addressing these gaps will help clarify how lampricide exposure influences the health, behaviour, and long-term survival potential of this imperiled species and inform more ecologically balanced sea lamprey management strategies.

Thesis overview

Effective management of invasive species requires balancing control efficacy with the protection of ecologically and culturally important native taxa. In the Great Lakes, the chemical control of sea lamprey using lampricides has been central to the recovery of native fisheries. However, the potential sub-lethal effects of these chemicals on non-target species such as lake sturgeon remain insufficiently understood. In this context, my thesis examines the olfactory and

physiological responses of yearling lake sturgeon exposed to lampricides under conditions representative of operational field treatments. Specifically, my thesis assesses how TFM and TFM-niclosamide exposures influence olfactory function (using EOG recordings) and stress physiology (using plasma cortisol and glucose) (Chapter 2). These endpoints are evaluated across multiple timepoints to capture both acute and recovery responses. I then integrate the experimental findings to consider patterns of physiological tolerance and vulnerability, interpret mechanistic relationships across endpoints, and discuss broader implications for conservation management and lampricide treatment practices (Chapter 3). Collectively, this thesis seeks to advance understanding of how lampricide exposure influences the physiology and sensory function of a sensitive native species, providing insights that can guide more ecologically informed and sustainable approaches to sea lamprey management in the Great Lakes.

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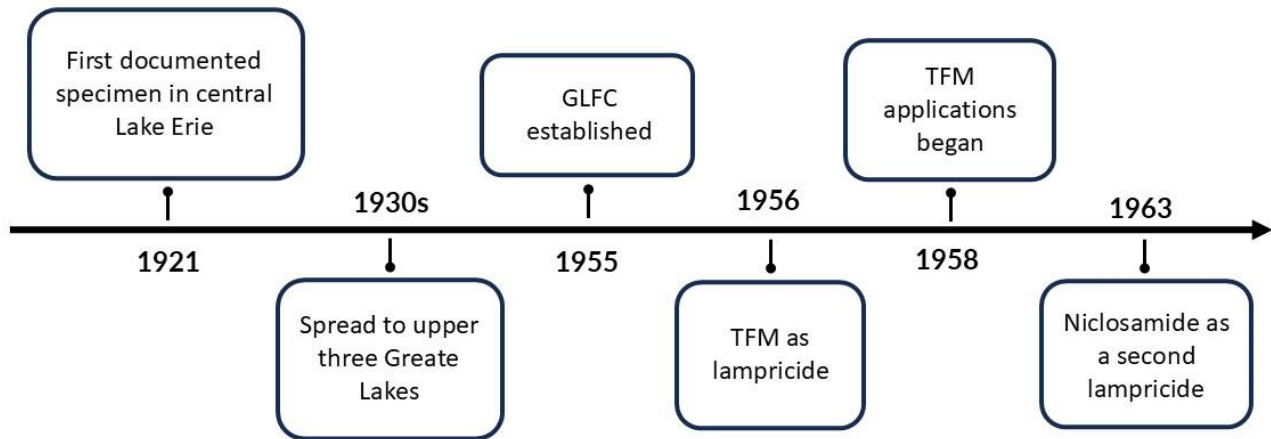


Figure 1.1. Historical timeline of sea lamprey (*Petromyzon marinus*) invasion and control in the Laurentian Great Lakes, illustrating major milestones in spread, governance (establishment of the Great Lakes Fishery Commission), and the adoption of lampricides (TFM and niclosamide) as management tools.

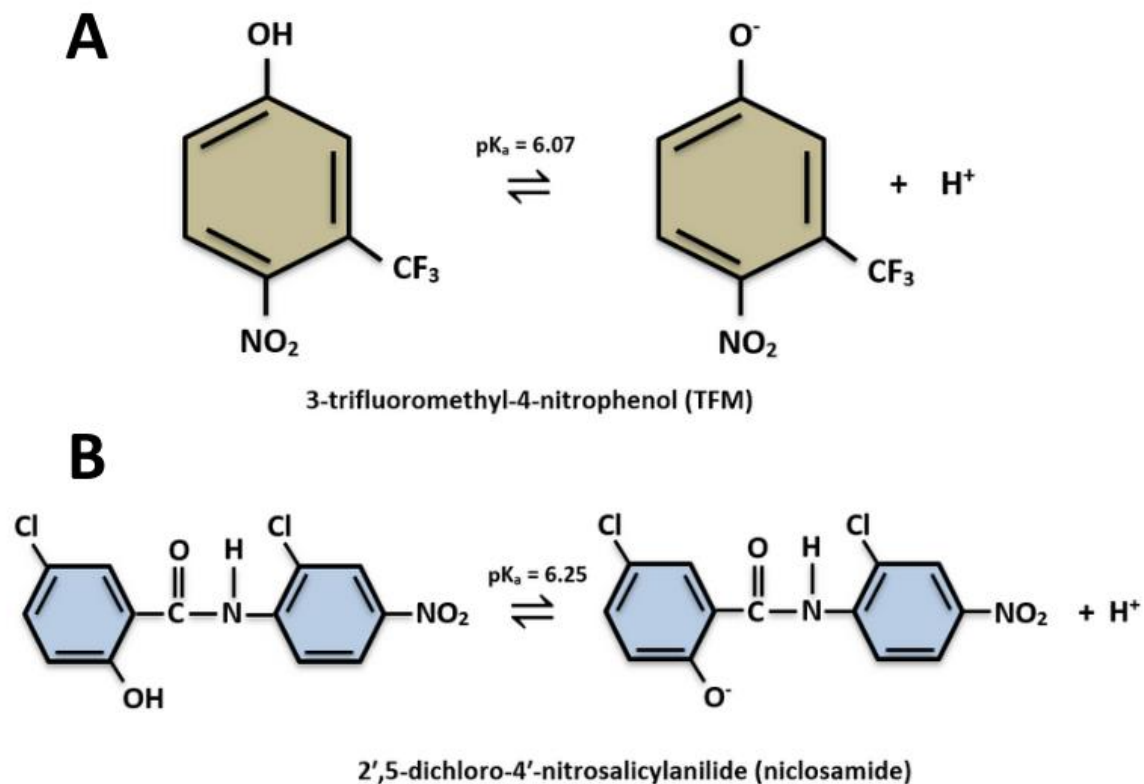


Figure 1.2. Chemical structures of (A) 3-trifluoromethyl-4-nitrophenol (TFM) and (B) 2',5-dichloro-4'-nitrosalicylanilide (niclosamide), showing their pH-dependent ionization. Both TFM (pKa = 6.07) and niclosamide (pKa = 6.25) are weak acids. At lower pH, each compound exists primarily in its un-ionized phenolic form, which is more lipophilic and diffusible across biological membranes. At higher pH, the ionized phenolate form predominates, reducing molecular diffusibility and uptake potential. Adapted from Wilkie et al. (2019).



Figure 1.3. Photo of juvenile lake sturgeon (*Acipenser fulvescens*)

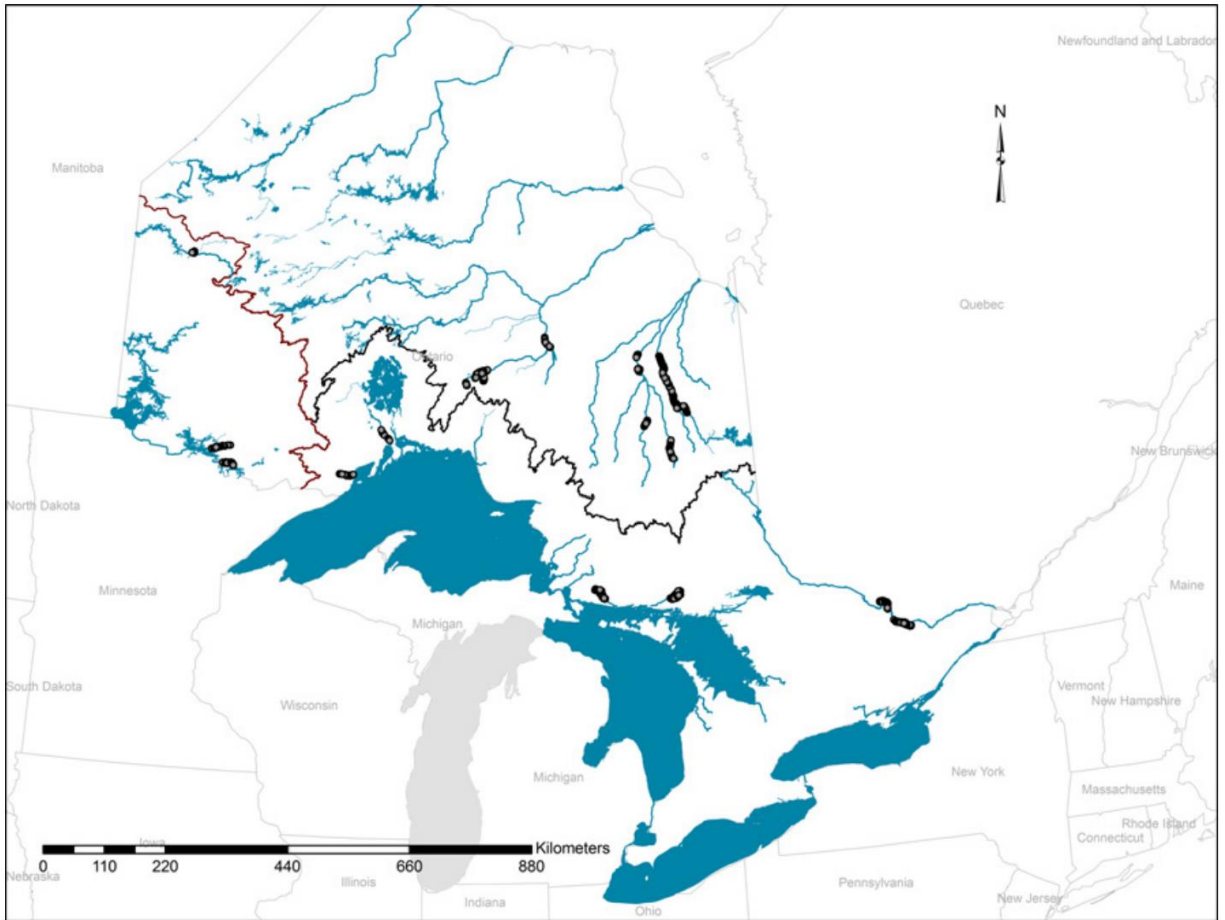


Figure 1.4. Distribution of lake sturgeon (*Acipenser fulvescens*) in Ontario across major drainage basins, adapted from Haxton et al. (2008).

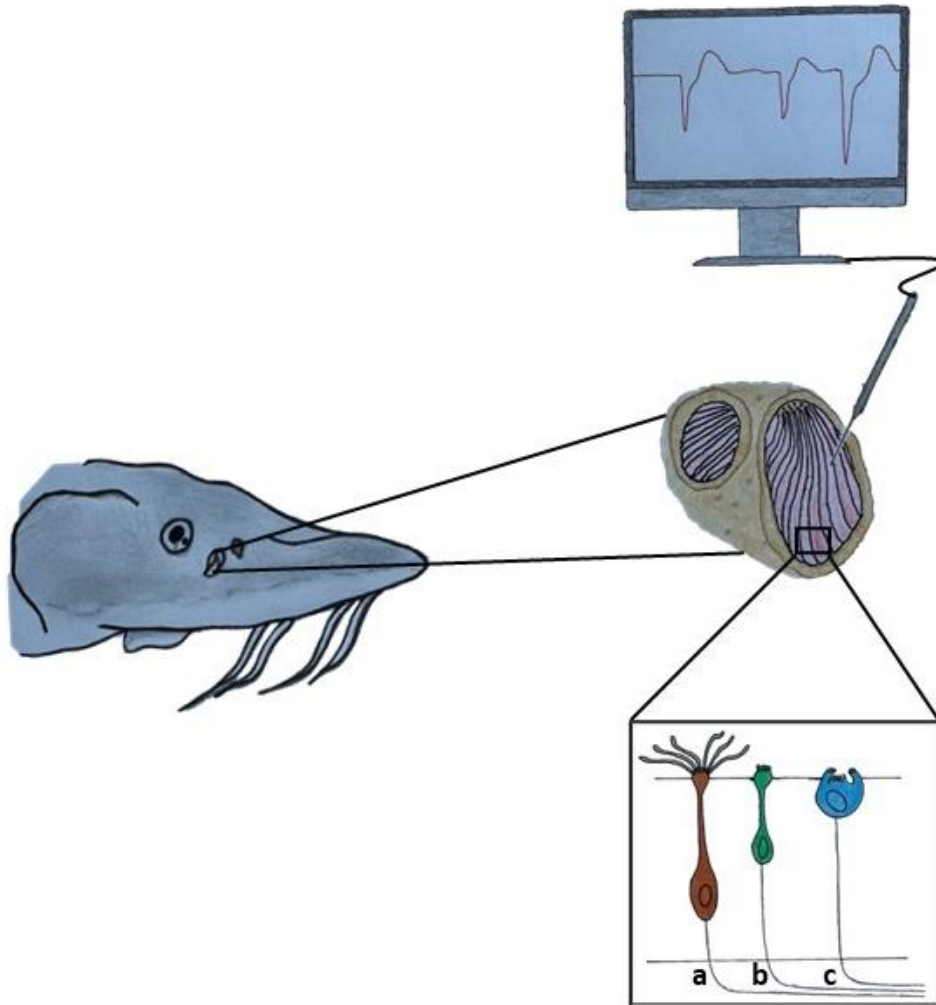


Figure 1.5. Conceptual schematic of the electro-olfactogram (EOG) recording setup and organization of the fish olfactory epithelium. The diagram illustrates the placement of the recording electrode over the olfactory rosette, signal acquisition and recording, and the major olfactory sensory neuron (OSN) types within the sensory epithelium, including (a) ciliated, (b) microvillous, and (c) crypt neurons. The morphology and relative arrangement of OSN types are based on published descriptions of teleost olfactory epithelia (Mori 2014).

Chapter 2 - An investigation of sublethal effects of lampricide exposure on olfaction and stress physiology of yearling lake sturgeon (*Acipenser fulvescens*)

Abstract

Lampricides are central to sea lamprey control in the Laurentian Great Lakes, yet their potential sublethal effects on non-target species continue to raise conservation concerns. Juvenile lake sturgeon are often considered vulnerable to lampricide exposure, but the sensitivity of life stages beyond young-of-the-year (YOY) under operational treatment conditions is not well resolved. We tested whether exposure to 3-trifluoromethyl-4-nitrophenol (TFM) or a TFM-1% niclosamide mixture alters olfactory responsiveness or stress-axis activity in yearling lake sturgeon. We exposed fish to lampricides at concentrations aligned with management practices for either 30 minutes or 12 hours, and physiological responses were evaluated after 30 minutes and 12 hours of exposure and after six days of depuration. Plasma cortisol and glucose were quantified to assess baseline endocrine status, the acute stress response, and potential subsequent recovery trajectories. Olfactory function was evaluated at 12 hours post exposure using electro-olfactography (EOG) by measuring responses of ciliated and microvillous olfactory sensory neurons to L-alanine and taurocholic acid. Across all endpoints, fish exposed to lampricide treatments did not differ from controls. Baseline cortisol levels, the capacity to mount an acute cortisol response to handling, and circulating glucose concentrations remained comparable among groups. Likewise, EOG amplitudes showed no treatment-related reductions, and a multivariate analysis confirmed no effect of lampricide exposure on combined olfactory responses across odorants. Together, these findings demonstrate that short-term, operationally relevant lampricide exposures under the tested

water chemistry conditions did not impair sensory or endocrine function in yearling lake sturgeon. These findings contrast with reports of olfactory impairment in YOY lake sturgeon and suggest that such sensory disruptions do not persist beyond the first year of growth under operationally relevant lampricide exposure conditions, with important implications for refining life-stage-specific risk assessments and conservation management.

Introduction

Sea lamprey (*Petromyzon marinus*) remain among the most consequential invasive species in the Laurentian Great Lakes, and the long-running control program centered on lampricide applications is widely credited with enabling the recovery of native fisheries and associated livelihoods (Lawrie 1970; Pearce et al. 1980; Smith and Tibbles 1980; Heinrich et al. 2003; Lavis et al. 2003; Siefkes 2017; Wilkie et al. 2019; Robinson et al. 2021). The principal chemical, 3-trifluoromethyl-4-nitrophenol (TFM), and its frequent co-application with niclosamide, are deployed in nursery streams to target larval lampreys during cyclical treatments that remove one or more cohorts at a time (Bills et al. 2003; McDonald and Kolar 2007). Selectivity arises from fundamental toxicokinetic differences between sea lamprey and most non-target fishes, particularly the limited capacity of sea lamprey to detoxify TFM via hepatic glucuronidation, resulting in prolonged internal exposure to the parent compound (Lech 1973; Lech 1974; Lech and Statham 1975; Kane et al. 1994; Clarke et al. 1991). At the toxicodynamic level, TFM acts as an uncoupler of mitochondrial oxidative phosphorylation, leading to disruption of ATP production when energetic demand cannot be met, a mechanism that underlies its lethality in sea lamprey larvae (Birceanu et al. 2009; Birceanu et al. 2011).

Birceanu et al. (2009) exposed larval sea lamprey to 4.6 mg L⁻¹ TFM (12-h LC₅₀) and reported an 80% decrease in brain glycogen, a 30% reduction in ATP, and an 80% reduction in phosphocreatine, along with a nine-fold increase in lactate, indicating a mismatch between ATP supply and demand due to increased reliance on glycolysis. Even with this success, the ecological reality of large-scale chemical control is that exposure extends beyond the target species, and sublethal effects in native fishes can occur under field-relevant conditions that vary with water chemistry, temperature, and local flow regimes (Bills et al. 2003; Boogaard et al. 2003; Wilkie et al. 2019; Wilkie et al. 2021).

Freshwater ecosystems already carry a heavy burden of interacting stressors that compromise biodiversity and ecosystem services at global scales, including hydrologic alteration, nutrient enrichment, chemical contamination, and climatic change (Dudgeon et al. 2006; Strayer and Dudgeon 2010; Carpenter et al. 2011; Reid et al. 2018). Within this context, risk assessments that focus only on lethality underestimate the cumulative consequences of transient but functionally meaningful physiological disruptions. Sublethal endpoints such as endocrine activation, metabolic disturbance, and sensory impairment can reduce foraging efficiency, elevate predation risk, and degrade reproductive success, thereby influencing survival trajectories and population resilience over time (Mommensen et al. 1999; Barton 2002; Derby and Sorensen 2008; Tierney et al. 2010). For lampricides specifically, experimental studies have shown that TFM can elevate plasma cortisol in trout, reduce tissue glycogen and ATP while increasing lactate, and impair olfactory function and behaviour in larval and juvenile sturgeon under realistic exposure scenarios (Birceanu et al. 2009; Birceanu et al. 2014; Sakamoto et al. 2016; Birceanu and Wilkie 2018; Ionescu et al. 2021). These outcomes are not

uniform across species or life stages, and they are modulated by environmental parameters such as pH and alkalinity that alter the proportion of unionized TFM and its uptake across the gill (Bills et al. 2003; Wilkie et al. 2021).

Lake sturgeon (*Acipenser fulvescens*) is long-lived, late-maturing, and characterized by low intrinsic population growth rates (Fortin et al. 1993; Vélez-Espino and Koops 2009). It has experienced substantial historical declines due to overharvest, habitat fragmentation, and pollution, and multiple populations remain at risk, including the Great Lakes-Upper St. Lawrence designatable unit that is Threatened in Canada (COSEWIC 2017). Early life stages face high natural mortality, and nursery habitats often overlap spatially and temporally with lampricide treatment zones, elevating the chance of exposure during sensitive developmental windows (Bruch et al. 2006; Sakamoto et al. 2016; Yoon et al. 2019; Pratt et al. 2021). Hepditch et al. (2019) reported that YOY lake sturgeon have 2-3 fold higher TFM uptake than yearling (age 1+) individuals, with susceptibility likely linked to small body size and elevated metabolic demand. Importantly, sturgeon can glucuronidate TFM at levels comparable to rainbow trout (LeClair and Wilkie 2014), so heightened sensitivity may reflect secondary factors (e.g., reduced glycogen stores, size-dependent exposure, metabolic constraints) rather than a deficiency in detoxification capacity (Boogaard et al. 2003; LeClair and Wilkie 2014; Sakamoto et al. 2016; Hepditch et al. 2019). For a species with slow recovery potential, even temporary physiological costs that depress growth or alter behaviour could scale to meaningful conservation consequences.

Among sublethal endpoints, olfaction is a central sensory modality with direct ecological relevance. Fish use odorant cues to locate prey, recognize predators, identify conspecifics, and

navigate to natal or suitable habitats for spawning and rearing (Dittman and Quinn 1996; Hara and Zielinski 2006; Derby and Sorensen 2008). The olfactory epithelium integrates signals across multiple receptor neuron classes called olfactory sensory neurons (OSNs) (Hansen and Zielinski 2005; Olivares and Schmachtenberg 2019). The electro-olfactogram (EOG) provides a tractable, quantitative measure of the summated receptor potential at the epithelial surface and can resolve changes in responsiveness across different stimulus categories when standardized odorants are used, such as taurocholic acid for ciliated OSNs and L-alanine for microvillous OSNs (Dew et al. 2014). Prior work by Sakamoto et al. (2016) in YOY lake sturgeon showed that TFM exposure depressed EOG amplitudes to both cue types and reduced attraction to food scents. Whether similar effects occur in older juveniles and whether they recover over short time frames is not well understood. In the same study, TFM exposure resulted in a reduced response to food cue in a behavioural trial and a lower feeding rate, supporting the use of EOG data as a surrogate for behaviour (Sakamoto et al. 2016).

Stress physiology provides a complementary integrative lens on organismal condition during and after chemical exposure, particularly through endocrine indicators such as cortisol. Baseline cortisol reflects an organism's endocrine status under non-stressed (i.e., acute) conditions and provides insight into metabolic readiness, osmoregulatory balance, and overall homeostatic condition (Crossin et al. 2016; Sopinka et al. 2016). Activation of the hypothalamic-pituitary-interrenal (HPI) axis in response to acute environmental stressors mobilizes energy and supports short-term survival, but sustained or repeated activation carries costs that can manifest as reduced growth, impaired immune function, and lowered reproduction success (Barton and Iwama 1991; Wendelaar Bonga 1997; Mommsen et al. 1999; Harper and Wolf

2009). Plasma cortisol is a widely used primary indicator of the stress axis, with downstream metabolic adjustments evident in circulating glucose and related metabolites (Barton 2002; Portz et al. 2006; Sopinka et al. 2016). In rainbow trout, TFM exposure transiently elevates plasma cortisol, yet the time course of response and recovery in sturgeon remains unclear and may differ with life stage, water chemistry, and the addition of niclosamide during stream applications (Birceanu and Wilkie 2018). Niclosamide is also used as a molluscicide and an anthelmintic, and acts as a potent mitochondrial uncoupler across taxa (Borowiec et al. 2022; Ionescu et al. 2025). Borowiec et al. (2022) demonstrated that niclosamide was 40-60 times more potent uncoupler of mitochondrial oxidative phosphorylation. Because sensory disruption and endocrine activation can co-occur, measuring both can reveal whether lampricide exposure imposes independent or interacting constraints on lake sturgeon behaviour and physiology.

Environmental factors strongly affect lampricide effectiveness and non-target responses. Lampricide performance and non-target risk vary with pH and alkalinity, which govern the protonation state of TFM and its gill bioavailability, and with hydraulic conditions that influence dosing precision and exposure duration during operational treatments (Bills et al. 2003; McDonald and Kolar 2007; Wilkie et al. 2021). These extrinsic factors intersect with intrinsic features of the organism, including body size and metabolic rate during early life stages (Bills et al. 2003; Hepditch et al. 2019). Yearling sturgeon occupy a transitional life stage between larval and older juvenile phases that likely brings changes in activity, habitat use, and physiological demand. This stage may therefore differ in sensitivity from the YOY age class most often studied. Understanding the magnitude and persistence of functional changes after realistic exposures in yearlings can therefore refine the risk profile assigned to lampricide

treatments and guide mitigation strategies such as scheduling, dosing adjustments, or post-treatment monitoring for sensitive reaches.

The present study addresses these gaps by pairing olfactory electrophysiology with endocrine and metabolic indicators in yearling lake sturgeon following exposure to TFM and a combination TFM with 1% niclosamide. We focus on odorants that index two major olfactory pathways with clear ecological relevance. L-alanine assays responsiveness of microvillous OSNs, while taurocholic acid (TCA) assays responsiveness of ciliated receptor populations (Hansen and Zielinski 2005; Derby and Sorensen 2008; Dew et al. 2014). We evaluate plasma cortisol as a primary index of HPI axis activation and blood glucose as a secondary metabolic response that reflects energetic mobilization and potential cost of coping with an environmental stressor (Mommsen et al. 1999; Barton 2002; Sopinka et al. 2016). By sampling across multiple time points that capture acute effects and short-term recovery, we assess whether any functional depression in olfaction is transient, whether endocrine activation is short-lived or persistent, and whether trajectories differ between TFM and TFM with niclosamide.

Based on prior evidence in rainbow trout and YOY lake sturgeon, we predicted that lampricide exposure would transiently reduce EOG amplitudes to both L-alanine and TCA, and would elevate plasma cortisol, with associated increases in blood glucose during the acute period (Sakamoto et al. 2016; Birceanu and Wilkie 2018; Ionescu et al. 2021). We further predicted that responses would partly recover within one week under our water chemistry conditions, but that the combination of TFM with niclosamide would produce larger or more persistent effects than TFM alone due to stronger disruption of mitochondrial respiration (Wilkie et al. 2019; Borowiec et al. 2022). Given that juvenile sturgeon may experience

metabolic and energetic constraints during lampricide exposure, we anticipated that even modest short-term impairments could be detectable at the level of physiology and sensory function (Boogaard et al. 2003; Le and Wilkie 2014; Hepditch et al. 2019).

Overall, evaluating olfactory sensitivity and stress physiology together in yearling lake sturgeon provides an integrated test of sublethal risk and resilience under exposure conditions chosen to approximate operational treatments. This study is designed to separate acute effects from early recovery, to compare two commonly used lampricide formulations, and to interpret functional consequences within the broader ecological and conservation context of a long-lived, at-risk species. By situating organismal responses within the environmental and management realities of the Great Lakes, the results aim to inform control strategies that continue to suppress sea lamprey while minimizing impacts on native biodiversity and the ecosystem services that freshwater systems provide (Dudgeon et al. 2006; Reid et al. 2018; Wilkie et al. 2019; Wilkie et al. 2021).

Methods

This study was conducted using yearling lake sturgeon housed at the Freshwater Restoration Ecology Centre (FREC) in LaSalle, Ontario, Canada. All experiments were carried out between 19 August and 4 September 2024 during a period of stable late-summer water temperatures that supported optimal husbandry conditions. The project was approved by the Algoma University Animal Care Committee (AUP 2024-CM-BD-Sturgeon-01) with reciprocal approval from the University of Windsor Animal Care Committee (AUP-24-08).

All fish originated from a single year-class cohort maintained at FREC for research and

restoration purposes. Wild lake sturgeon gametes were originally collected from the St. Clair River (Port Huron, Michigan, USA) between 5 and 15 June 2023, and juveniles were reared for approximately three months at the Genoa National Fish Hatchery (Genoa, Wisconsin, USA). During this period, individuals were implanted with 8-mm, 134.2-kHz passive integrated transponder (PIT) tags for permanent identification. At the time of experimentation, fish were 14–15 months old ($n = 122$; mass: 48.70 ± 1.81 g; total length: 25.7 ± 0.3 cm).

Fish were held in three 850-L circular fiberglass tanks (density: ~ 2.97 g/L) supplied by a shared recirculating system with UV sterilization and 10% daily water exchange. Water temperature was maintained at 13-16 °C under a 12 h light : 12 h dark photoperiod controlled by programmable LED lighting. Fish were fed a commercial sinking pellet diet formulated for sturgeon.

All lampricide exposures were conducted in six independent 80-L tanks filled to 50 L with aerated, dechlorinated water drawn directly from the sturgeon housing recirculating system. Each tank was equipped with an air bubbler to maintain dissolved oxygen and gentle circulation.

At each time period, treatments were randomly assigned among the six tanks, with five fish per tank: two control tanks (untreated rearing water), two tanks dosed with TFM, and two tanks dosed with TFM combined with niclosamide (TFM-N). Lampricide stock solutions were prepared fresh daily using analytical-grade reagents (TFM: TCI America; niclosamide: Sigma-Aldrich). Exposure concentrations were determined using the sea lamprey MLC (Minimum Lethal Concentration) calculator based on measured background water chemistry (alkalinity: 96.6 mg/L CaCO_3 ; pH 7.60), resulting in final concentrations of 1.4 mg/L TFM (TFM-only) and 0.7

mg/L TFM + 7.5 µg/L niclosamide (TFM–N). At the end of each exposure period, fish were immediately processed according to the relevant sampling protocol (blood sampling or EOG; see below). Total length and mass, PIT tag number, treatment assignment, sampling date and time, and tank ID were recorded each time a fish was handled. Water temperature and dissolved oxygen were measured before and after exposures to verify consistency among tanks. One unique set of fish were sampled after 30-min of exposure, whereas other fish were held in 12-hr exposures and then resampled after a 6-day depuration period in their home tanks (Figure 2.1). Twelve-hour exposure trials were initiated at 21:00 and concluded at 09:00 the following morning to maintain consistent diel conditions across trials.

Cortisol and glucose

We assessed cortisol and glucose at three sampling times: (1) 30-min exposure (2) 12-h exposure (3) 6-days post-exposure recovery (Figure 2.1). At each timepoint, we measured both baseline and stress induced components of stress physiology.

Stress induction protocol

To determine baseline circulating levels of cortisol and to initiate and assess activation of the HPI axis, we used a standardized handling protocol. Prior to the start of full experimentation, a subset of fish (n = 15) was sampled directly from their holding tanks. Fish were individually netted gently without chasing or disruption to other conspecifics and exposed to air for 10 s, matching the typical transfer duration used during experimental handling. Baseline blood samples were collected within 3 min of capture from the caudal vein on the ventral surface just posterior to the anal fin using a 0.3-mL heparinized syringe fitted with a 29-

gauge needle. During blood sampling, fish were held dorsally in a shallow wooden trough partially filled with fresh water that covered the gills and were supported on a sponge to minimize stress and body curvature. After baseline sampling, each fish was transferred into a cooler filled with fresh water and supplied with an air bubbler for a 30-min period to allow the primary cortisol response to develop. A second blood sample was then collected from a slightly anterior site using the same procedure to characterize HPI-axis reactivity. Following blood sampling, fish were returned to their holding tanks. All handling during blood sampling was performed using moist bare hands to maintain mucous integrity.

30-minute exposure (immediate response)

To determine whether lampricide exposure led to an immediate change in circulating cortisol, n=45 sturgeon were exposed for 30 min to either control water, TFM, or TFM-N (three replicate tanks per treatment with n=5 fish per tank). Prior to introducing fish, freshly prepared TFM and TFM-N stock solutions were added directly to the designated exposure tanks, and water was gently mixed to ensure complete dispersion. Each tank was equipped with an air bubbler for the full duration of holding.

Fish were netted from their holding tanks, transferred directly into the exposure tanks, and the tanks were immediately covered to prevent escape and create a dim environment similar to holding tanks. Fish remained undisturbed for the 30-minute exposure period. At the end of the exposure, a single blood sample was collected from each fish as described above.

12-hour exposure

To determine the effect of an extended lampricide exposure, we exposed n=45 sturgeon

per group to control water, TFM, or TFM-N for 12 h (three tanks per treatment group with n= 5 fish per tank). Immediately after exposure, fish were sampled for baseline cortisol within 3 min of capture, followed by a second sample after a 30-min holding period in aerated rearing water to assess ability to mount a stress response to a standardized stressor.

6-day depuration

Following the 12-h exposure described above, all fish were transferred to their home (holding) tanks maintained under standard conditions for six days. Baseline and post-handling blood samples were repeated to evaluate the extent of physiological recovery following chemical exposure.

Blood processing

All whole blood samples were held on ice and processed for glucose within 1-hour of sampling using Contour Next test strips (Ascensia Diabetes Care), previously validated for lake sturgeon (Galloway et al. 2025). The remaining whole blood was separated by centrifugation and plasma was stored for cortisol analyses at -20°C until analysis. Plasma cortisol concentrations were quantified using a commercial ELISA kit (Cayman Chemical, Kit 500360) following the manufacturer's instructions and previously validated for lake sturgeon (Galloway et al. 2025). Briefly, plasma samples were diluted 1:20 in assay buffer and run in duplicate. For each plate, 50 μL of diluted sample, 50 μL of AChE tracer, and 50 μL of monoclonal antibody were added to wells and incubated overnight at 4°C . Plates were washed five times with 200 μL wash buffer and developed with 200 μL Ellman's reagent for 90 min on an orbital shaker (450 rpm). Absorbance was measured at 412 nm using a spectrophotometer plate reader. Final

cortisol concentrations (ng/mL) were calculated by averaging the duplicate absorbance-derived concentrations and multiplying by the dilution factor. A small proportion of samples (7.1%) fell below the detection limit and were assigned the minimum quantifiable value (i.e., assay sensitivity of 0.60 ng/mL). Intra-assay variability, calculated across duplicate wells for all samples, averaged 8.2%, and inter-assay variability, calculated from kit control wells across plates, was 8.64%.

Electro-olfactography (EOG)

Electro-olfactograms (EOGs) were used to evaluate olfactory sensitivity after 12-h exposure (n=9 control, n=5 TFM, n=4 TFM-N; Figure 2.1). Fish were anesthetized in a 4-L bath containing 160 mg/L MS-222 (Syndel), buffered to pH 7.0 with sodium bicarbonate (Thermo Fisher Scientific). Once immobilized, fish were wrapped in moist paper towel, positioned ventrally on a custom holder affixed to the recording apparatus, and grounded to minimize electrical noise. Anaesthesia was maintained in gill perfusion water at a concentration of 80 mg/L MS-222 (buffered with sodium bicarbonate) throughout recording.

The right olfactory rosette was accessed and a glass microelectrode (~350- μ m tip diameter) filled with an electrolyte solution (0.9% w/v NaCl + 4% w/v gelatin) was placed between olfactory lamellae within the olfactory epithelium. A reference electrode was placed on the skin adjacent to the olfactory chamber. Odorants were delivered using a gravity-fed perfusion system at a constant flow rate. Stimuli included L-alanine (10^{-3} M; Thermo Fisher Scientific), taurocholic acid (TCA; 10^{-4} M; Thermo Fisher Scientific), and a blank water control (Azizishirazi et al. 2013; Dew et al. 2014; Sakamoto et al. 2016). Stimulus solutions were prepared fresh before each trial and delivered for 3 s with 60-s inter-stimulus intervals to

prevent sensory adaptation.

Each fish received at least three replicates per odorant, and responses were averaged for analysis. Signals were amplified, digitized, and stored for offline processing. After recordings, fish were placed into fresh recovery water in 5 L tubs until gill movement, tail movement, and equilibrium were fully restored before being returned to the holding system. All handling was performed using moist gloves to maintain mucous integrity.

Statistical analyses

All statistical analyses were performed in R (v. 4.5.0; R Core Team 2023) using the *lme4*, *lmerTest*, and *performance* packages. All figures were generated using GraphPad Prism (v.10.6.1; GraphPad Software, LLC).

Cortisol

Cortisol analyses were conducted using linear mixed-effects models (LMMs), with model structure tailored to the specific physiological question being tested. Treatment, mass, and bleed time were modeled as fixed effects, while tank ID was included as a random effect in all cortisol models to account for shared housing. Julian date was included as an additional random effect for the 30-min, 12-h and 6-days timepoint to account for day-to-day variation in sampling conditions.

All cortisol values were log-transformed to meet the assumptions of normality. Model assumptions were assessed using the `check_model()` function from the *performance* package (Lüdecke et al. 2021), including posterior predictive plots, residual vs. fitted distributions, Q-Q plots for residual and random effects, and multicollinearity diagnostics. These assessments

confirmed that model assumptions were met, and no data transformations beyond the selected log models were required. Values exceeding three standard deviations from the mean were flagged as outliers and removed from the analyses, resulting in the removal of three data points.

Separate LMMs were fit for each timepoint in accordance with the physiological question being tested. Baseline and post-handling cortisol were compared prior to any lampricide exposure to confirm that the standardized handling and blood-sampling procedure reliably elicited an acute endocrine response. The 30 min dataset contained only baseline cortisol values and was analyzed to determine whether brief exposure to TFM or TFM-N induced an immediate change in circulating cortisol relative to untreated controls. For the 12 h and 6 days datasets, baseline models tested whether lampricide exposure altered the baseline (pre-stress) endocrine state. Further, to assess stress reactivity at these same timepoints, an Δ cortisol metric was calculated as the difference between an individual's post-stress cortisol concentration and its corresponding baseline value (Δ cortisol = post-stress – baseline). This value reflects the magnitude of the endocrine response to the handling stressor, representing physiological reactivity rather than resting endocrine state. Δ cortisol values at 12 h and 6 days were analyzed using LMMs with the same fixed and random structures used for baseline models, allowing treatment effects on both baseline cortisol level and magnitude of change under stress to be evaluated independently. For all models, post-hoc treatment effects were evaluated using Type III ANOVA tables, and Satterthwaite-corrected degrees of freedom were used for inference.

Glucose

Glucose analyses followed the same overall LMM framework as cortisol to evaluate basal glucose levels and stress-induced glucose responses across exposure periods. Treatment, mass, and bleed time were included as fixed effects, while tank ID and Julian date were modeled as random intercepts. Models were fitted using both raw and log-transformed glucose, and the preferred scale was selected based on AIC comparison. In all cases, the log-transformed glucose models showed superior fit ($\Delta AIC > 2$).

Model assumptions were assessed using the same approach, `check_model()` from the performance package (Lüdecke et al. 2021), which confirmed that the model assumptions were met, and no additional transformations were required beyond log scaling. Values exceeding three standard deviations from the mean were flagged as outliers and removed from the analyses.

Separate LMMs were fitted to test whether lampricide treatments altered glucose responses across sampling periods. To confirm that the standardized handling procedure reliably elicited a metabolic stress response, baseline and stress glucose concentrations prior to any exposure were analyzed. To evaluate whether lampricide exposure induced an acute effect independent of handling stress, baseline glucose levels at 30 min post-exposure were analyzed. For the 12 h and 6 days datasets, baseline models tested whether lampricide exposure altered resting metabolic state, while separate post-stress models evaluated whether exposure affected the capacity to mount a stress-induced glucose response. For all glucose models, treatment effects were evaluated using Type III ANOVA tables with Satterthwaite-corrected degrees of freedom.

Electro-olfactography (EOG)

Blank-corrected EOG amplitudes for TCA and L-alanine were analyzed to evaluate whether exposure to TFM or TFM-N altered olfactory sensitivity after 12 h of exposure. Prior to hypothesis testing, multivariate and univariate normality were assessed using the Henze-Zirkler and Anderson-Darling tests implemented in the *MVN* package (Korkmaz et al. 2014). Both odorants satisfied normality assumptions (Henze-Zirkler $p = 0.36$; TCA $p = 0.94$; L-alanine $p = 0.77$).

To test for treatment effects on overall olfactory responsiveness, a multivariate analysis of variance (MANOVA) was conducted with treatment group as the fixed factor and blank-corrected amplitudes to TCA and L-alanine as the dependent variables. Because the MANOVA indicated no significant multivariate effect of treatment, no additional univariate post-hoc analyses were performed. All EOG statistical analyses were conducted in R (v. 4.5.0; R Core Team 2023) using functions from the *stats* and *MVN* packages.

Results

Cortisol

Cortisol concentrations were significantly higher (~nine-fold) following the standardized handling stressor compared to baseline (Type III LMM, treatment: $F_{1,20.9} \approx 74.6$, $p < 0.001$; Figure 2.2; Table 2.1). Circulating baseline cortisol concentrations measured immediately after the 30-min exposure did not differ significantly among treatment groups (Type III LMM, $F_{2,2.99} \approx 1.92$, $p = 0.62$; Figure 2.4; Table 2.2). At 12 h post-exposure, baseline cortisol concentrations did not differ among treatments ($F_{2,37} \approx 0.98$, $p = 0.38$; Figure 2.6; Table 2.3). Similarly, Δ cortisol

values, did not differ among groups ($F_{2,35} \approx 1.24$, $p = 0.30$; Figure 2.6; Table 2.4). After six days of recovery, baseline cortisol levels did not differ across treatments ($F_{2,2.95} \approx 0.44$, $p = 0.68$; Figure 2.8; Table 2.5). Similarly, Δ cortisol responses at this timepoint were not significantly different among treatment groups ($F_{2,2.49} \approx 0.18$, $p = 0.84$; Figure 2.8; Table 2.6).

Glucose

Plasma glucose concentrations increased following the handling stressor relative to baseline (Type III LMM, treatment: $F_{1,24} \approx 12.15$, $p = 0.0019$; Figure 2.3; Table 2.1). At the 30-min timepoint, baseline glucose concentrations did not differ significantly among treatment groups ($F_{2,2.98} \approx 0.12$, $p = 0.89$; Figure 2.5; Table 2.2). At 12 h post-exposure, treatment effects were not detected in baseline glucose ($F_{2,37} \approx 0.37$, $p = 0.69$; Figure 2.7; Table 2.3) or stress induced glucose responses ($F_{2,0} \approx 0.14$, $p = 0.99$; Figure 2.7; Table 2.4). After six days of depuration, no treatment effects were detected in baseline glucose ($F_{2,36.01} \approx 0.45$, $p = 0.64$; Figure 2.9; Table 2.5) or glucose responses ($F_{2,2.0} \approx 0.48$, $p = 0.67$; Figure 2.9; Table 2.6).

Electro-olfactograms

A MANOVA evaluating combined olfactory responses across treatment groups showed no effect of lampricide exposure (Pillai's trace = 0.044, $F_{4,3} = 0.17$, $p = 0.95$; Figure 2.10; Figure 2.11). Because there was no significant multivariate effect, further univariate comparisons were not pursued.

Discussion

Lampricides play a critical role in suppressing invasive sea lamprey populations in the

Laurentian Great Lakes, yet their use in streams that also support juvenile lake sturgeon has raised concerns regarding potential sublethal effects on this species (Boogaard et al. 2003; Sakamoto et al. 2016; O'Connor et al. 2017; Hepditch et al. 2019; Pratt et al. 2021). Most available research has focused on larval and YOY sturgeon, while the sensitivity of older juvenile stages under operational treatment conditions remains poorly understood (Sakamoto et al. 2016; Hepditch et al. 2019; Ionescu et al. 2021). This study experimentally evaluated whether exposure to TFM or a TFM with 1% niclosamide mixture, at environmentally relevant concentrations, affects olfactory sensitivity or stress-axis activity in yearling lake sturgeon. To address this question, we assessed electro-olfactogram (EOG) response amplitudes to L-alanine and taurocholic acid, representing odorants that primarily stimulate microvillous and ciliated olfactory sensory neurons (OSNs) respectively (Hansen and Zielinski 2005; Dew et al. 2014). We also quantified plasma cortisol and glucose to assess baseline endocrine status, the magnitude of acute stress responses to handling, and subsequent recovery across multiple time points representing acute exposure (30 minutes), extended exposure (12 hours), and early recovery (six days). By sampling across these time points, the study was designed to determine whether any sensory or endocrine effects were transient, persistent, or absent under the applied exposure conditions.

Exposure to TFM and TFM-N did not alter olfactory responsiveness, baseline cortisol concentrations, the capacity to mount an acute cortisol response, or the corresponding glucose levels in yearling lake sturgeon under the water chemistry and field-relevant dosing conditions used in this study. Olfactory sensitivity remained intact across the two OSNs classes tested. Baseline cortisol and glucose remained comparable across treatments, indicating that

lampricide exposure did not disrupt resting endocrine or energetic state. These findings contrast with several reports of sublethal lampricide effects in young-of-the-year (YOY) sturgeon and other fishes, and suggest that yearlings may be less sensitive to operationally relevant lampricide exposures than the YOY age class. It is also notable that individual variability in physiological stress responsiveness was substantial across fish, and such natural variation can reduce the detectability of small treatment effects when the incremental stress load imposed by lampricides is minor relative to handling or background conditions.

Olfactory integrity in yearling lake sturgeon

No depression of EOG amplitude occurred in response to either odorant, indicating that lampricide exposure did not impair responsiveness of either ciliated or microvillous OSNs. This result stands in contrast to prior observations in YOY sturgeon, where TFM exposure reduced EOG amplitudes to both L-alanine and TCA and suppressed food-search behaviour (Sakamoto et al. 2016). Several factors may explain the absence of similar effects in yearlings. Hepditch et al. (2019) showed that TFM uptake was 2-3 times higher in YOY lake sturgeon compared to age-1 fish across all water chemistry conditions tested, and suggested that this may partly reflect higher mass-specific metabolic rates in smaller individuals. They also reported that TFM uptake decreased as alkalinity increased from low ($\sim 50 \text{ mg L}^{-1}$ as CaCO_3) to moderate levels ($\sim 150 \text{ mg L}^{-1}$), before plateauing at higher alkalinity ($\sim 250 \text{ mg L}^{-1}$), indicating that both age and water chemistry jointly influence internal exposure. Wilkie et al. (2021) also noted that differences in gill structure and the extent of gill microenvironment acidification may contribute to the heightened sensitivity of YOY lake sturgeon to TFM under certain alkalinity conditions,

potentially allowing internal exposure in smaller fish to approach or exceed that of sea lamprey.

The absence of lampricide effects in both OSN classes suggests that epithelial excitability and receptor-level sensory transduction were not disrupted to a degree sufficient to alter the summated EOG signal. It is also important to note that EOG captures only peripheral receptor-level responsiveness; more subtle alterations in central olfactory processing or olfactory-mediated behaviour could occur without being reflected in EOG amplitudes. However, Sakamoto et al. (2016) found reduced EOG amplitude to coincide with altered behavioural responses to food cues, suggesting that peripheral sensory disruption is functionally meaningful when it does occur. No such peripheral impairment was detected in the present study, indicating that yearlings maintained sensory responsiveness to both food- and bile salt-related odorants under the exposure conditions used. These findings indicate that, under moderate alkalinity and near-neutral pH, yearling olfactory function at the level of the epithelium is not detectably affected by exposure concentrations representative of operational stream treatments, contrasting with reports of sensory disruption in younger life stages.

Lack of endocrine and energetic responses

Cortisol and glucose dynamics also showed no evidence of lampricide-induced perturbation. Handling stress reliably elevated cortisol (almost nine-fold) and modestly increased glucose (1.5-fold), confirming physiological responsiveness of the HPI axis. Importantly, neither TFM nor TFM-N altered resting cortisol concentrations, indicating no disruption to basal cortisol regulation. Likewise, the capacity to mount an acute cortisol response following handling stress remained intact, demonstrating that the HPI axis was

responsive and able to perform its normal mobilization function. Circulating glucose also did not differ across treatments and timepoints, suggesting that lampricide exposure likely did not interfere with some of the metabolic adjustments associated with either baseline or stress-induced endocrine activity. This outcome is notable because TFM exposure has been shown to disrupt energy metabolism in other species and at earlier life stages in lake sturgeon, particularly at higher exposure concentrations than those used in the present study. In larval sea lamprey, exposure to 4.6 mg L⁻¹ TFM (12-h LC₅₀) resulted in a mismatch between ATP production and ATP consumption, leading to large decreases in brain and liver glycogen, reduced ATP and phosphocreatine, and ultimately neural arrest and death (Birceanu et al. 2009). Follow-up work demonstrated that TFM acts as a protonophore in both sea lamprey and rainbow trout, uncoupling mitochondrial oxidative phosphorylation, dissipating the proton motive force, and impairing ATP synthesis when tested at 50 µmol L⁻¹ TFM (Birceanu et al. 2011). Similarly, juvenile lake sturgeon exposed to 4.7 mg L⁻¹ TFM (the larval sea lamprey 9-h LC_{99.9} concentration) experienced 50-80 % reductions in brain and liver ATP, phosphocreatine, and glycogen, increased lactate, and a slight metabolic acidosis (~0.1 pH unit), leading the authors to conclude that TFM causes metabolic disturbances that can impair physiological performance and, in some cases, result in mortality (Ionescu et al. 2021).

By contrast, rainbow trout exposed to TFM at concentrations typically used in lampricide applications showed no adverse physiological effects and exhibited a significant capacity to detoxify TFM via glucuronidation (LeClair and Wilkie 2014). The same study demonstrated that lake sturgeon were also capable of biotransforming TFM and generating TFM–glucuronide at levels similar to rainbow trout, although they were tested at lower

concentrations, and possible toxic effects at higher doses could not be ruled out (LeClair and Wilkie 2014). These findings suggest that metabolic disruption tends to occur when TFM is present at concentrations high enough to interfere with oxidative ATP production (such as LC_{50} or $LC_{99.9}$), whereas fish exposed to operational field concentrations and capable of conjugating TFM do not necessarily exhibit overt physiological impairment. In this context, the absence of changes in resting cortisol, Δ cortisol, or circulating glucose in yearling lake sturgeon in the present study, where concentrations were 1.4 mg L^{-1} TFM or 0.7 mg L^{-1} TFM + $7.5 \text{ } \mu\text{g L}^{-1}$ niclosamide, aligns with an exposure scenario that did not trigger the type of energetic disturbance observed under higher lampricide concentrations in sea lamprey, rainbow trout, or juvenile lake sturgeon.

The absence of measurable effects across olfactory and endocrine endpoints indicates that, under the tested exposure conditions, lampricides did not induce sufficient physiological strain to alter peripheral sensory function or circulating stress indicators in yearling lake sturgeon. Nevertheless, these findings do not preclude the occurrence of more subtle biochemical adjustments that may not manifest in circulating cortisol, glucose, or EOG amplitudes. For example, Madison et al. (2013) demonstrated that rainbow trout infected with *Cryptobia salmositica* exhibited clear physiological disturbance, including altered expression of metabolic and steroidogenic genes and reduced interrenal cortisol synthesis, despite minimal changes in plasma cortisol levels. This highlights that tissue-level or molecular shifts may occur without producing detectable systemic endocrine responses, and underscores the value of integrating tissue-based metabolic or molecular endpoints into future assessments of lampricide effects.

Life-stage, toxicokinetic, and environmental context of lampricide sensitivity

Lampricide toxicity is strongly modulated by water chemistry, especially pH and alkalinity, which determine the proportion of unionized TFM available for gill uptake (Bills et al. 2003; Wilkie et al. 2021). The alkalinity (≈ 97 mg/L CaCO_3) and pH (≈ 7.6) in this study likely reduced the internal TFM burden relative to conditions associated with heightened sensitivity. Moreover, regardless of pH or alkalinity, Hepditch et al. (2019) observed a 2-3-fold higher TFM uptake in YOY than yearling lake sturgeon. The comparatively lower uptake in yearlings under these exposure conditions may partly explain the absence of measurable effects on sensory and endocrine endpoints. Because pH and alkalinity were measured once at the start of the experiment to determine target concentrations, rather than daily, slight variations in source-water chemistry over time may have further lowered effective lampricide potency relative to predictions from the MLC calculator. Any such drift would place fish toward a shallower region of the dose-response curve, reducing the likelihood of detecting sublethal effects.

Life-stage differences are an increasingly recognized consideration in non-target risk assessments. YOY lake sturgeon have shown sensitivity to TFM, including behavioural changes and disrupted olfaction (Sakamoto et al. 2016; Ionescu et al. 2021), though responses are not uniform across sublethal endpoints and study conditions (Middaugh et al. 2014). Our results indicate that this heightened sensitivity does not necessarily persist into the yearling stage under moderate alkalinity and near-neutral pH. This distinction is ecologically relevant, as yearlings may occupy a wider range of stream habitats and do not always reside in the shallow depositional zones where TFM concentrations tend to peak during treatments (Haxton 2011). However, their spatial dispersion does not eliminate exposure risk, particularly in low-alkalinity

or low-pH nursery tributaries where TFM uptake is amplified (Bills et al. 2003; Hepditch et al. 2019; Wilkie et al. 2021). Rather than implying that yearlings are unaffected in all circumstances, the findings support a more nuanced interpretation: sensitivity appears reduced compared to YOY fish, but remains conditional on environmental context, exposure duration, and physiological state.

Implications for non-target risk and lampricide management

My findings suggest that yearling lake sturgeon are relatively unlikely to experience sublethal sensory and endocrine disruption under the tested conditions. In the context of Great Lakes management, this outcome indicates that the continued use of TFM-based lampricides under water chemistry regimes similar to those evaluated here likely will not produce measurable sublethal impairments to stress physiology and olfaction in yearling lake sturgeon. However, this apparent lack of effect must be interpreted conservatively. Sensitivity to TFM has been shown to increase in low-pH and low-alkalinity waters, where a higher proportion of TFM occurs in the unionized form and gill uptake is enhanced (Hepditch et al. 2019; Wilkie et al. 2021). Temperature can also influence toxicity in a non-linear manner, with Bouffard (2025) demonstrating that 1+ lake sturgeon experienced the highest mortality and internal TFM accumulation at 16 °C, and greater tolerance at both 8 and 20 °C.

Moreover, YOY stages remain considerably more vulnerable, and natural populations contain a mixture of life stages that may experience different exposure intensities. Although no effects were detected on the olfactory and endocrine endpoints measured here, the present study was not designed to evaluate subtle behavioural changes, central sensory processing, or

long-term energetic and fitness consequences. Future research would benefit from extending these evaluations beyond peripheral olfactory and circulating endocrine measures to include behavioural assessments, such as odour-guided trials, which have previously identified functional impairment in YOY sturgeon (Sakamoto et al. 2016). Incorporating tissue-level metabolic indicators, including glycogen, ATP, and lactate concentrations, would further help determine whether more subtle energetic adjustments occur even when circulating hormones and sensory responsiveness appear unchanged.

By integrating sensory and physiological endpoints, this work provides a refined understanding of non-target risk for an at-risk native species central to Great Lakes conservation. Adjustments to treatment timing or localized mitigation may still be warranted in reaches with high densities of young sturgeon. Nonetheless, our results indicate that in yearlings, lampricide exposures approximating field conditions are unlikely to impair olfactory function or stress-axis performance. Although subtle behavioural or biochemical effects cannot be ruled out, the absence of treatment-related changes across multiple functional systems indicates that yearlings are unlikely to experience significant sublethal impairment at operational lampricide concentrations under the tested conditions. These findings underscore the importance of life-stage-specific approaches to risk assessment and highlight the value of physiologically grounded evaluations when balancing invasive species control with the protection of native biodiversity.

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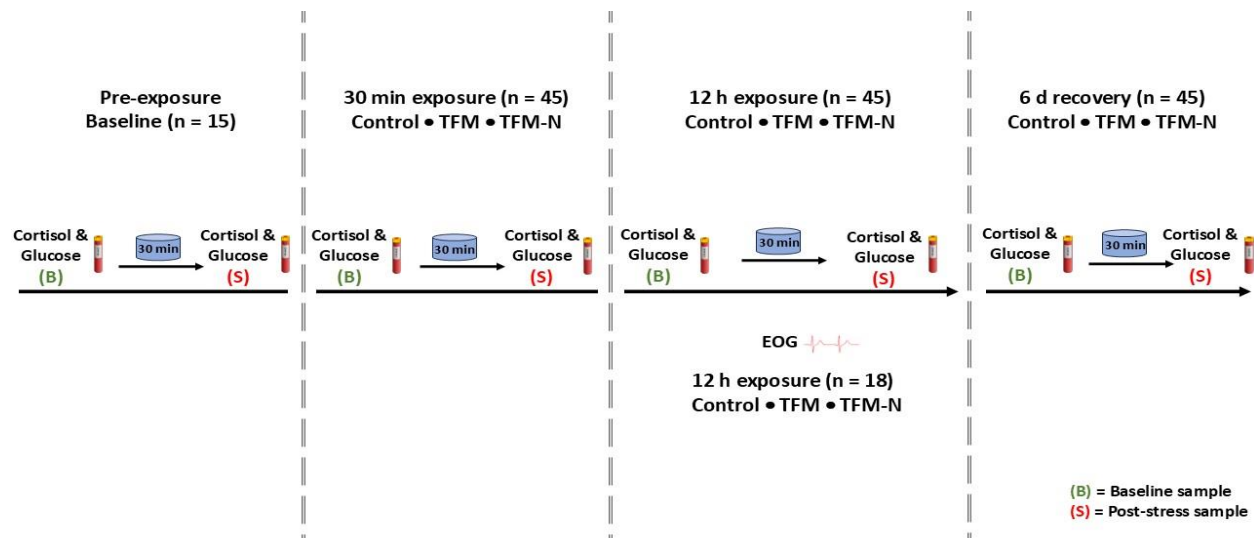


Figure 2.1. Overview of sampling design for cortisol, glucose, and electro-olfactogram (EOG) measurements in yearling lake sturgeon. Independent groups of fish were sampled at pre-exposure baseline, 30 min exposure, and 12 h exposure across three treatments (Control, TFM, TFM-N). At each timepoint, blood samples were collected at baseline (B) and 30 min after handling stress (S) to quantify plasma cortisol and glucose. The 12 h exposure cohort was then transferred to clean water and resampled after a 6 d recovery period. EOG recordings were conducted at 12 h exposure on a subset of fish.

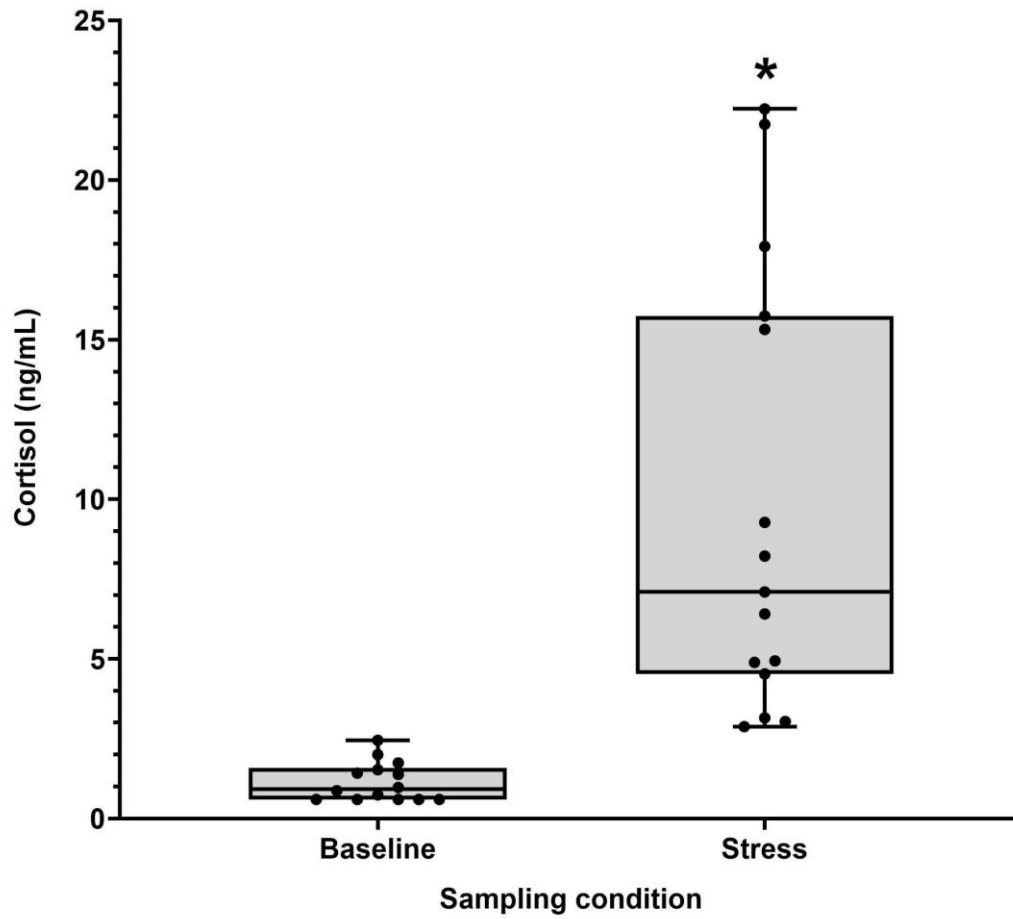


Figure 2.2. Plasma cortisol concentrations before (Baseline) and after (Stress) the standardized handling and blood-sampling procedure on Day 0. Boxplots show median and interquartile range, with whiskers representing the minimum and maximum values; individual points denote measurements from each fish. Asterisks indicate statistically significant differences ($p < 0.05$).

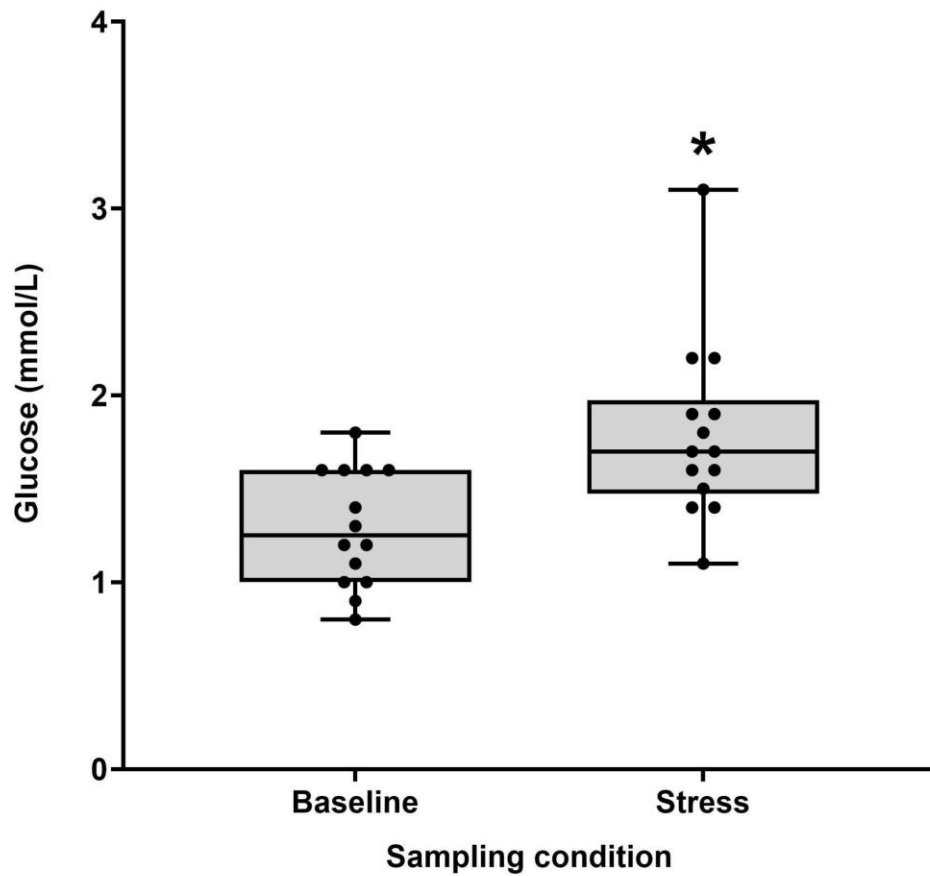


Figure 2.3. Whole blood glucose concentrations measured before (Baseline) and after (Stress) the standardized handling and blood-sampling procedure. Boxplots show median and interquartile range, with whiskers spanning the minimum and maximum values; each point represents an individual fish. Asterisks indicate statistically significant differences ($p < 0.05$).

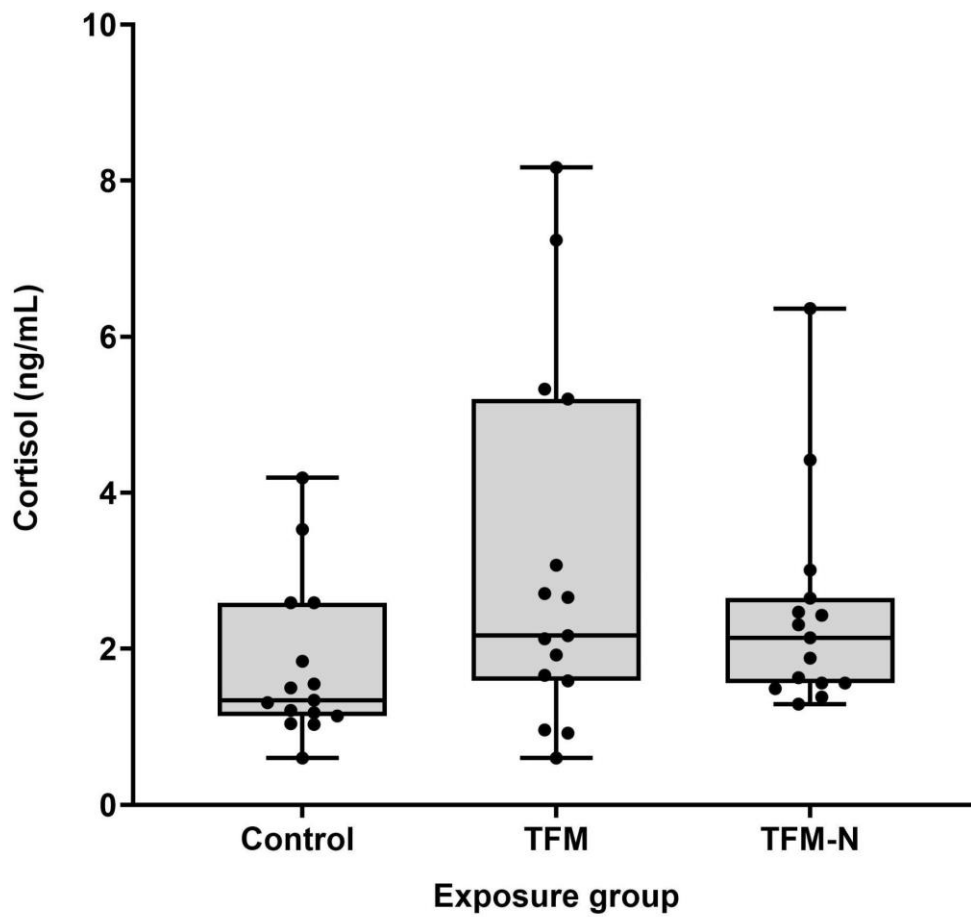


Figure 2.4. Plasma cortisol concentrations in fish exposed for 30 minutes to control water, TFM, or TFM with 1% niclosamide (TFM-N). Boxplots display median and interquartile range, with whiskers showing the minimum and maximum values and points representing individual fish.

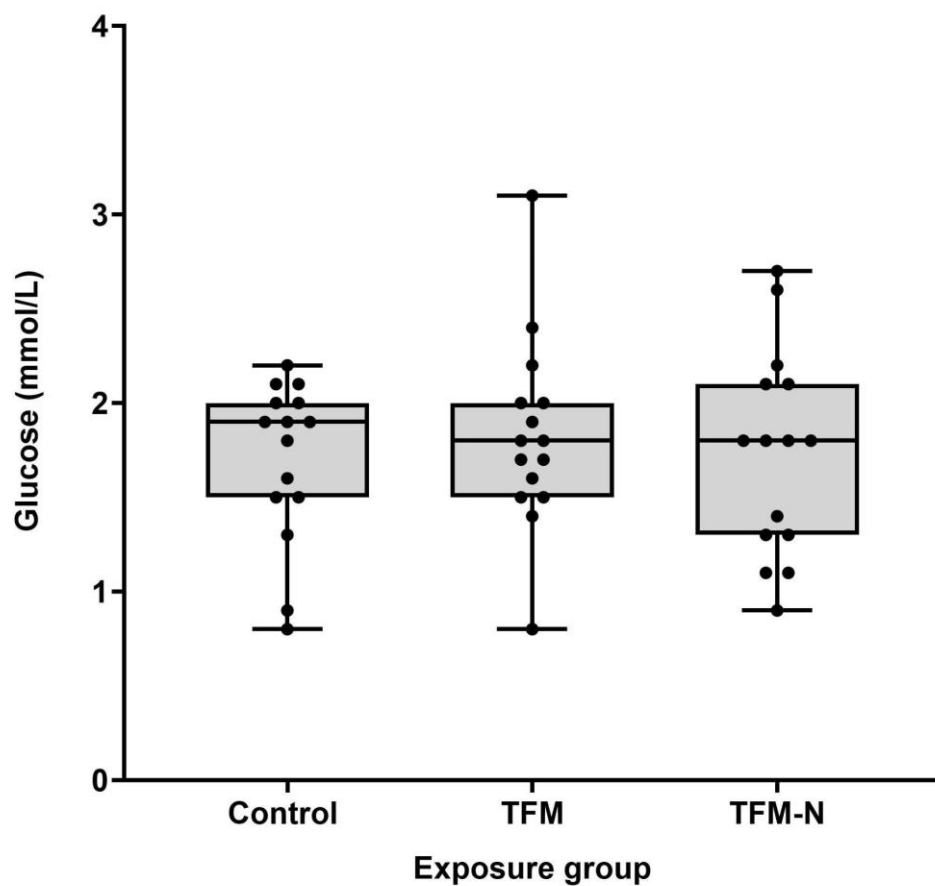


Figure 2.5. Whole blood glucose concentrations after 30-minute exposure to control water, TFM, or TFM-N. Boxplots represent median and interquartile range, with whiskers showing minimum and maximum values; each point denotes an individual fish.

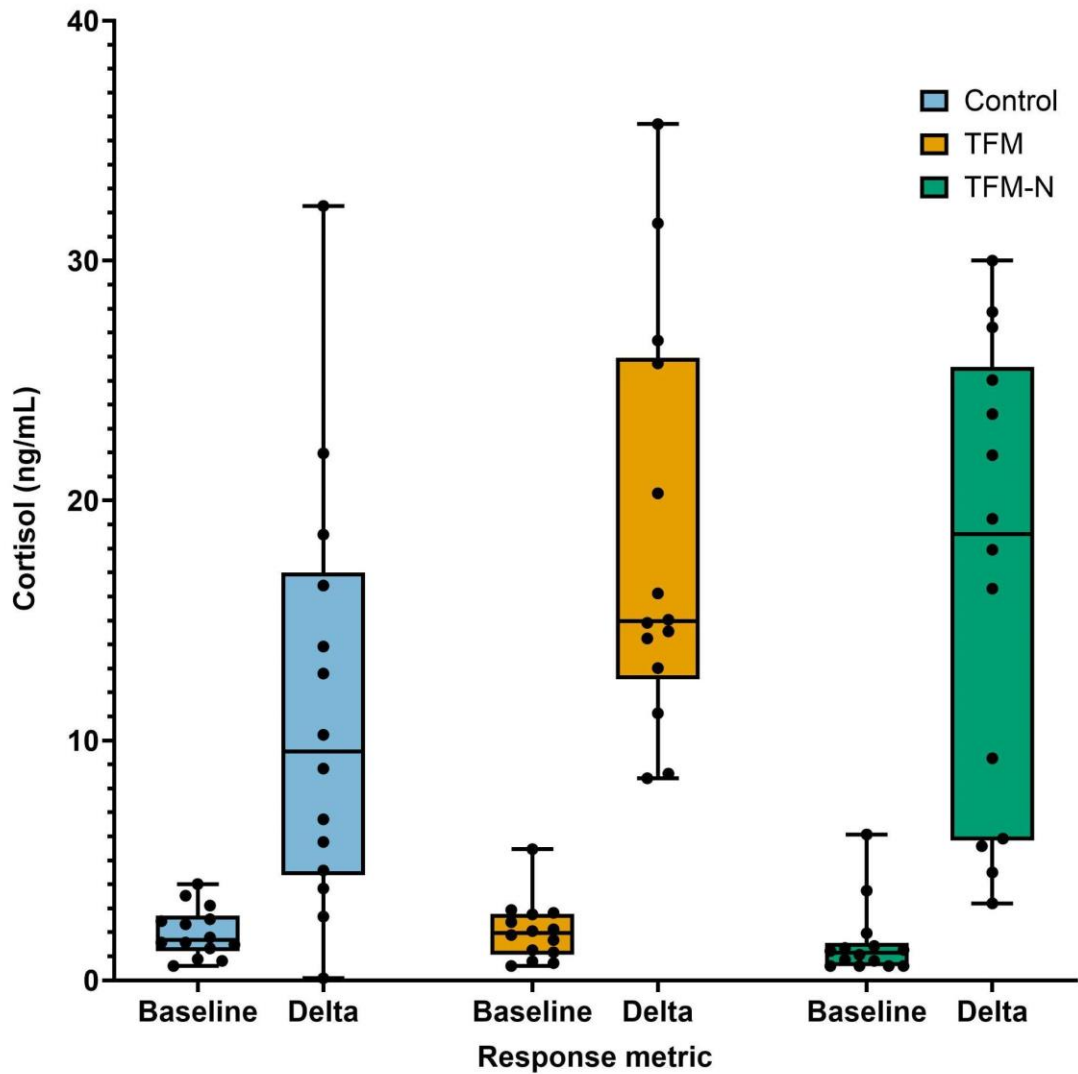


Figure 2.6. Plasma cortisol concentrations measured 12 hours after exposure to control water, TFM, or TFM-N. Left panels show baseline cortisol; right panels show the stress-induced change in cortisol (Δ cortisol = post-stressor - baseline). Boxplots show median and interquartile range, with whiskers showing minimum and maximum values; points represent individual fish.

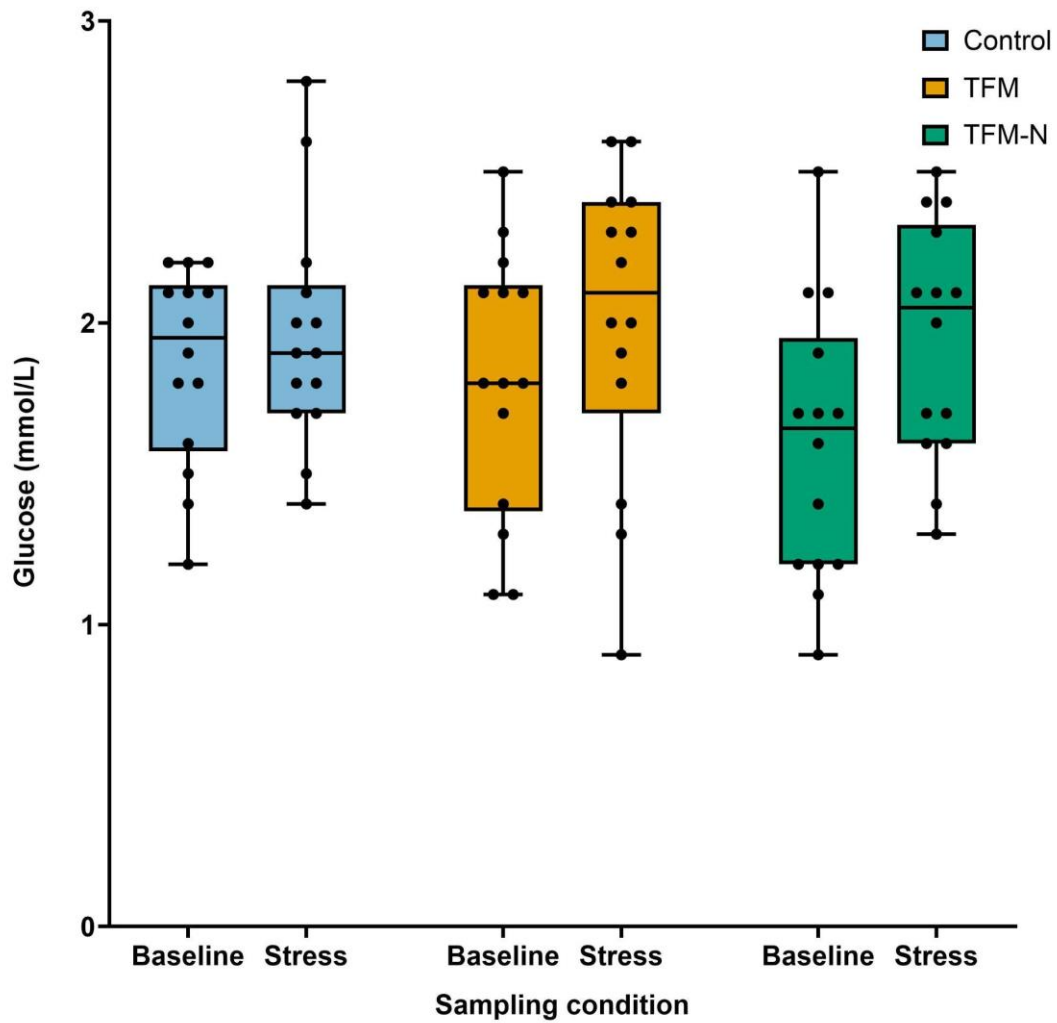


Figure 2.7. Whole blood glucose concentrations measured at baseline and after an acute stressor 12 hours following exposure to control water, TFM, or TFM-N. Boxplots indicate median and interquartile range, whiskers show the minimum and maximum values; points represent individual fish.

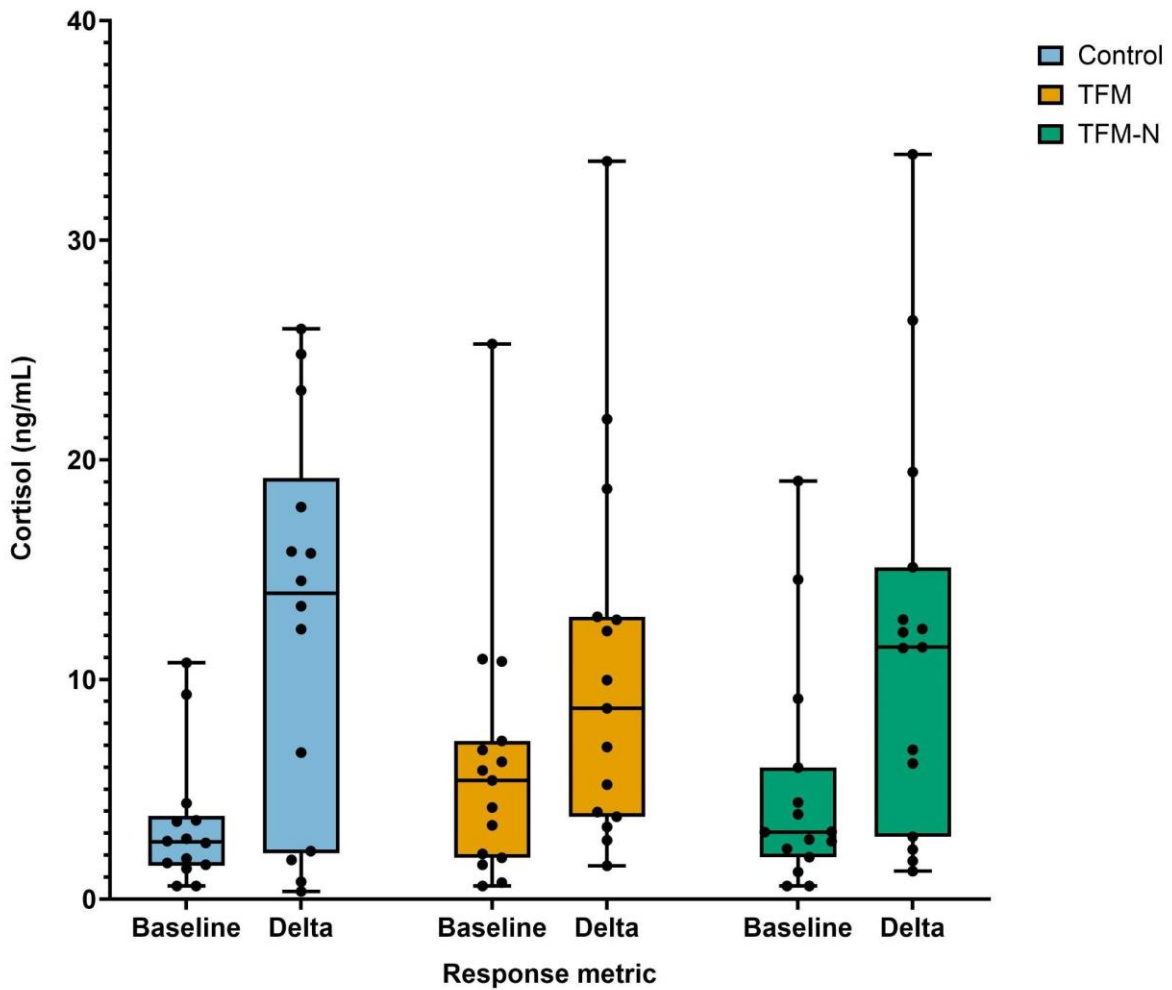


Figure 2.8. Plasma cortisol concentrations measured six days after exposure to control water, TFM, or TFM-N. Left panels show baseline cortisol, and right panels show the stress-induced change in cortisol (Δ cortisol = post-stressor - baseline). Boxplots display median and interquartile range, with whiskers depicting minimum and maximum values; each point represents an individual fish.

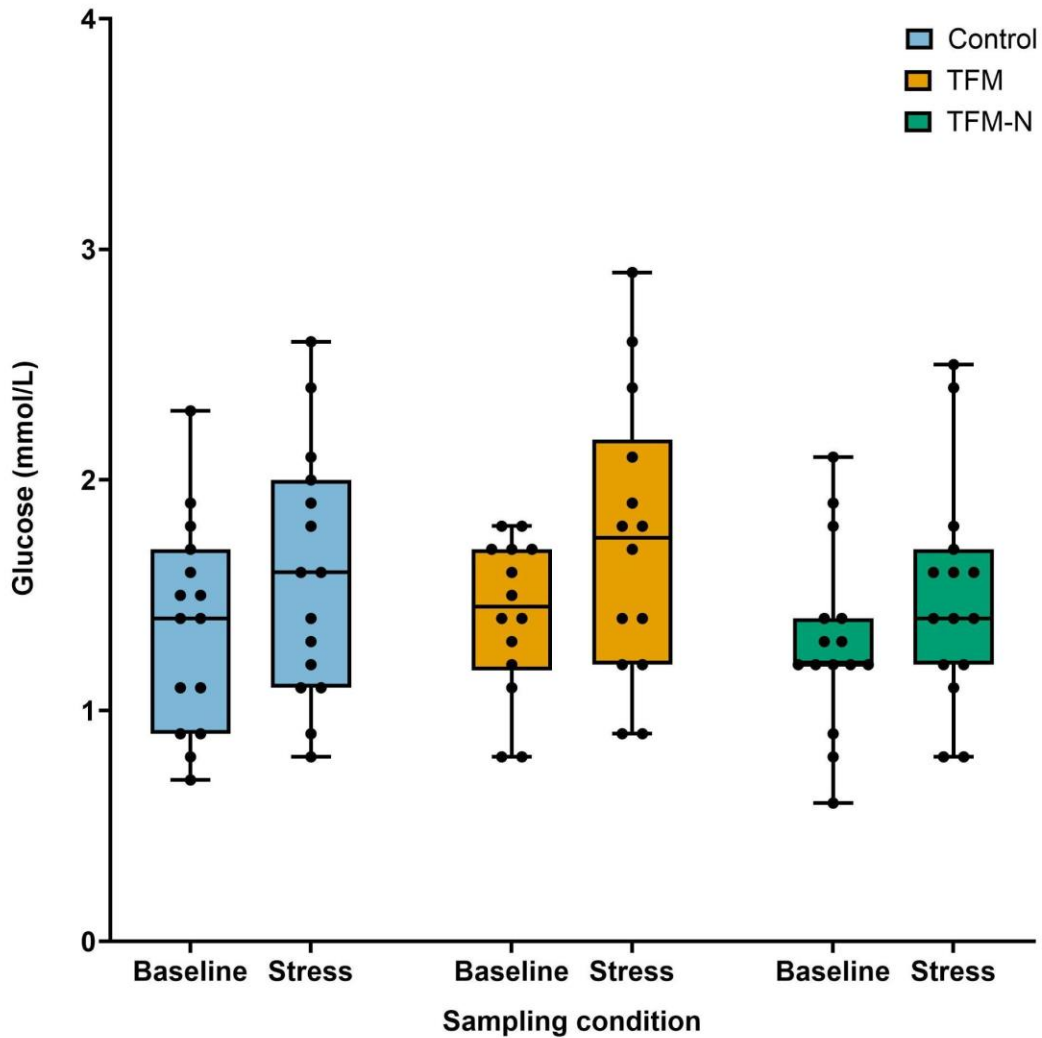


Figure 2.9. Whole blood glucose concentrations measured six days after exposure to control water, TFM, or TFM-N. Boxplots show median and interquartile range, whiskers represent the minimum and maximum values, and points indicate individual fish.

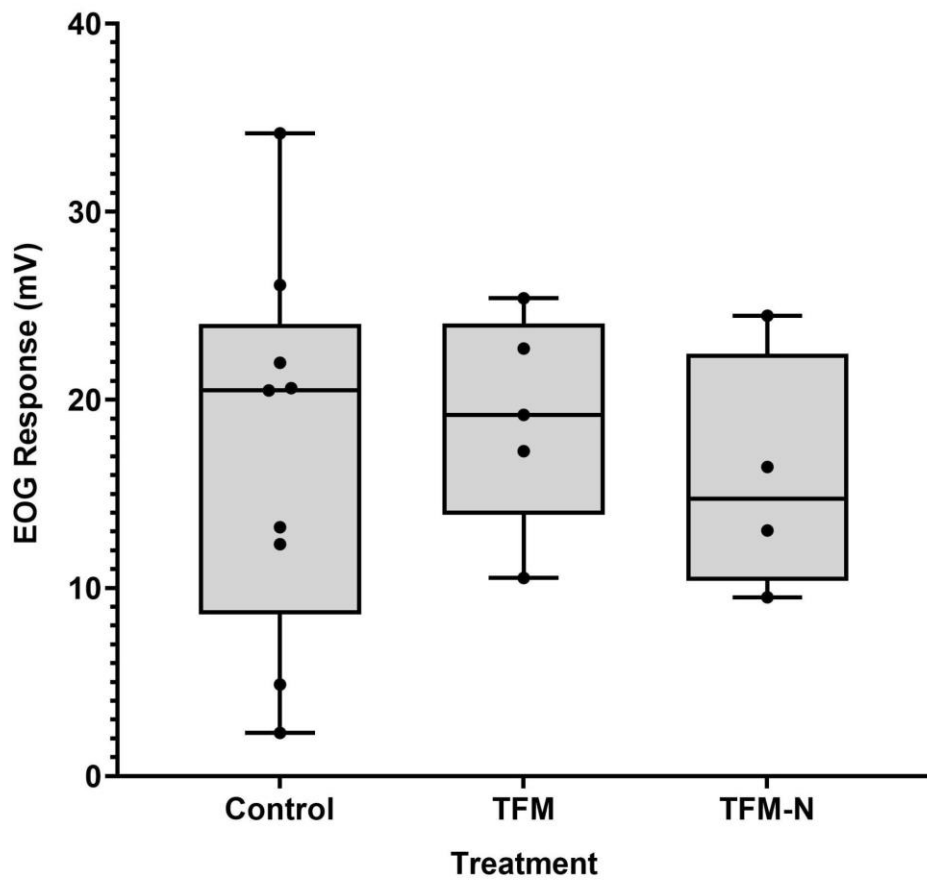


Figure 2.10. Blank-corrected electro-olfactogram (EOG) amplitudes evoked by TCA, representing ciliated olfactory sensory neuron responses, measured 12 hours after exposure to control water, TFM, or TFM-N. Boxplots show median and interquartile range, whiskers represent the minimum and maximum values, and overlaid points correspond to individual fish.

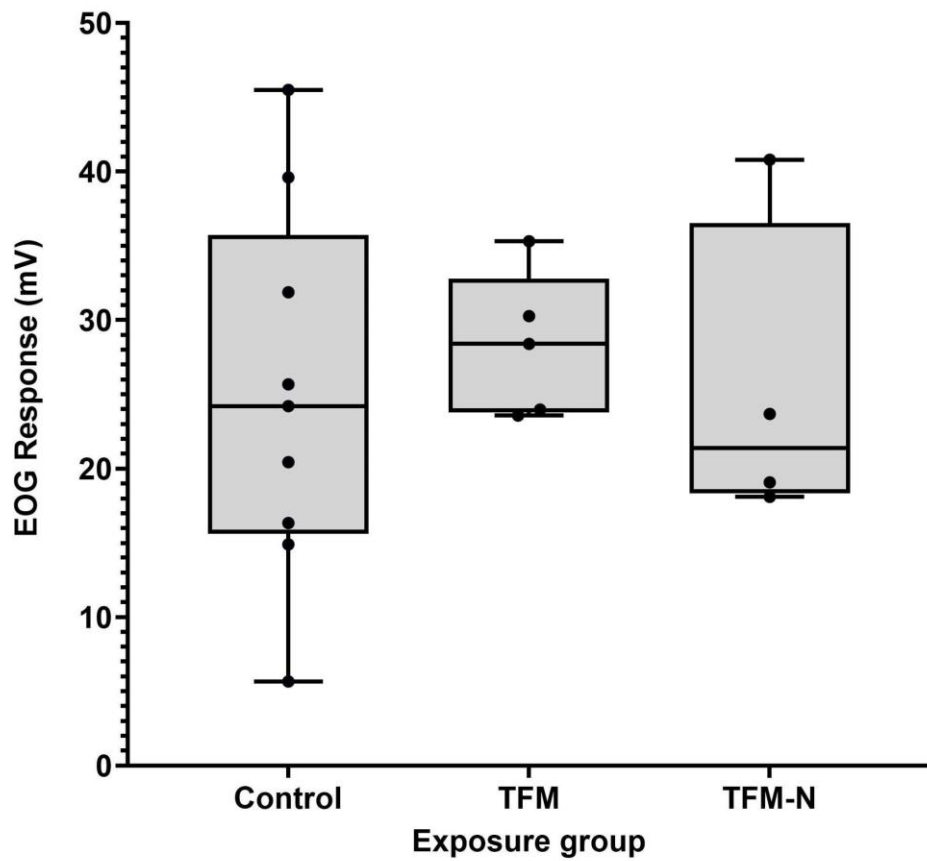


Figure 2.11. Blank-corrected EOG amplitudes evoked by L-alanine, representing microvillous olfactory sensory neuron responses, assessed 12 hours after exposure to control water, TFM, or TFM-N. Boxplots depict median and interquartile range with whiskers showing the minimum and maximum; individual points indicate single-fish measurements.

Table 2.1. Type III ANOVA (from linear-mixed-effects models) testing treatment effects with covariates of bleed time and mass on cortisol and glucose concentrations prior to lampricide exposure in yearling lake sturgeon

	Df (Num, Den)	F value	<i>P</i>
Cortisol			
Treatment	1, 20.93	74.64	<0.001
Mass	1, 18.27	1.67	0.21
Bleed time	1, 22.92	0.66	0.42
Glucose			
Treatment	1, 24	12.15	0.0019
Mass	1, 24	3.29	0.08
Bleed time	1, 24	0.57	0.46

Table 2.2. Type III ANOVA (from linear-mixed-effects models) testing treatment effects with covariates of bleed time and mass on cortisol and glucose concentrations after 30 min lampricide exposure in yearling lake sturgeon

	Df (Num, Den)	F value	<i>P</i>
Cortisol			
Treatment	2, 2.99	0.55	0.62
Mass	1, 37.60	0.76	0.39
Bleed time	1, 37.98	0.54	0.47
Glucose			
Treatment	2, 2.97	0.12	0.89
Mass	1, 37.90	19.32	< 0.001
Bleed time	1, 38.44	1.14	0.29

Table 2.3. Type III ANOVA (from linear-mixed-effects models) testing treatment effects with covariates of bleed time and mass on baseline cortisol and glucose concentrations after 12 hour lampricide exposure in yearling lake sturgeon

	Df (Num, Den)	F value	<i>P</i>
Cortisol			
Treatment	2, 37	0.98	0.38
Mass	1, 37	0.00	0.95
Bleed time	1, 37	0.89	0.35
Glucose			
Treatment	2, 37	0.37	0.69
Mass	1, 37	3.60	0.07
Bleed time	1, 37	1.47	0.23

Table 2.4. Type III ANOVA (from linear mixed-effects models) testing treatment effects with covariates of mass and bleed time on stress-induced cortisol (Δ cortisol = post-stressor value - baseline value) and stress glucose concentrations after 12-hour lampricide exposure in yearling lake sturgeon.

	Df (Num, Den)	F value	<i>P</i>
Δ Cortisol			
Treatment	2, 35	1.24	0.30
Mass	1, 35	3.96	0.05
Bleed time	1, 35	0.55	0.47
Glucose			
Treatment	2, 0	0.14	0.99
Mass	1, 35	19.02	< 0.001
Bleed time	1, 35	0.09	0.76

Table 2.5. Type III ANOVA (from linear-mixed-effects models) testing treatment effects with covariates of bleed time and mass on baseline cortisol and glucose concentrations 6 days post lampricide exposure in yearling lake sturgeon

	Df (Num, Den)	F value	<i>P</i>
Cortisol			
Treatment	2, 2.95	0.44	0.68
Mass	1, 34.55	0.12	0.73
Bleed time	1, 35.25	5.16	0.03
Glucose			
Treatment	2, 36.01	0.45	0.64
Mass	1, 36.54	12.95	< 0.001
Bleed time	1, 36.58	1.40	0.24

Table 2.6. Type III ANOVA (from linear mixed-effects models) testing treatment effects of bleed time and mass on stress-induced cortisol (Δ cortisol = post-stressor value - baseline value) and stress glucose concentrations 6 days post lampricide exposure in yearling lake sturgeon.

	Df (Num, Den)	F value	<i>P</i>
Δ Cortisol			
Treatment	2, 2.49	0.18	0.84
Mass	1, 28.22	0.48	0.49
Bleed time	1, 29.12	0.01	0.94
Glucose			
Treatment	2, 2.00	0.48	0.67
Mass	1, 32.22	17.98	< 0.001
Bleed time	1, 34.31	0.09	0.77

Chapter 3 - General discussion and conclusions

Summary of principal findings

Lampricide treatments remain indispensable for controlling invasive sea lamprey in the Laurentian Great Lakes, and their continued use underpins the protection and recovery of native fisheries and associated ecosystem functions (Lawrie 1970; Heinrich et al. 2003; Siefkes 2017). However, concerns persist regarding potential sublethal effects of lampricide treatments on non-target fishes, especially those of conservation concern such as lake sturgeon, which often co-occur in treated nursery streams during vulnerable juvenile stages (O'Connor et al. 2017; Dobiesz et al. 2018; Pratt et al. 2021). While several studies have investigated the effects of TFM and TFM-niclosamide mixtures on larval and young-of-the-year (YOY) lake sturgeon, their relevance to yearlings under operational exposure conditions remains uncertain, and little is known about whether functional endpoints such as olfactory responsiveness and endocrine stress performance are affected in yearlings (Sakamoto et al. 2016; Hepditch et al. 2019; Ionescu et al. 2021). There is a gap in our ability to evaluate lampricide vulnerability at other life stages which constrains evidence-based management decisions regarding treatment timing, exposure predictability, and non-target risk evaluation.

The overarching objective of this thesis was to determine whether environmentally realistic exposures to TFM or TFM-niclosamide mixtures disrupt sensory or physiological function in yearling lake sturgeon. Through an experimental design incorporating validated electro-olfactogram (EOG) methods and repeated measurements of cortisol and glucose across multiple sampling periods, this work sought to evaluate whether exposure to TFM or a TFM

with 1% niclosamide (TFM-N) mixture alters olfactory sensitivity, resting endocrine status or the capacity to mount an acute stress response under field-relevant conditions. Across all measured physiological and sensory endpoints, no treatment effects were detected.

The standardized handling protocol (i.e., induction of stress with handling) elicited a significant cortisol increase and a modest rise in glucose concentration, confirming that the hypothalamic-pituitary-interrenal (HPI) axis and associated metabolic pathways were responsive to acute stress and that the sampling framework was sufficient to detect physiological disturbance. During the acute exposure interval (30 minutes), cortisol concentrations did not differ among fish exposed to control water, TFM, or TFM-N, and glucose values likewise remained comparable across treatments. After 12 hours, baseline cortisol, the handling-induced change in cortisol (Δ cortisol), baseline glucose, and stress-induced glucose all remained indistinguishable among groups, indicating that neither baseline physiological function nor acute stress responsiveness were altered by lampricide exposure. Endocrine measures also did not differ among treatments six days following exposure, demonstrating that no treatment-related physiological effects emerged during the later sampling interval.

Electro-olfactogram (EOG) recordings yielded a similarly consistent pattern of insensitivity to lampricides at the peripheral sensory level. Blank-corrected responses to taurocholic acid and L-alanine were variable among individuals but did not differ among control, TFM, and TFM-N treatments. The multivariate model incorporating responses to both odorants indicated that olfactory function was unaffected by lampricide exposure when assessed 12 hours after dosing.

Taken together, these results demonstrate that, under moderate alkalinity and near-neutral pH, neither short-term TFM nor TFM-N exposure disrupted baseline cortisol production, acute cortisol responsiveness, circulating glucose, or peripheral olfactory sensitivity in yearling lake sturgeon. This contrasts with findings in YOY sturgeon, where Sakamoto et al. (2016) reported marked reductions in olfactory response amplitudes to L-alanine, taurocholic acid, and food-related cues following exposure to ecologically relevant TFM concentrations, accompanied by altered feeding behaviour and increased activity. Similarly, Ionescu et al. (2021) demonstrated that exposure to the larval sea lamprey LC_{99.9} TFM concentration caused substantial depletion of ATP, glycogen, phosphocreatine, and glucose in YOY sturgeon tissues, indicating pronounced metabolic disturbance during early-life stages. These comparisons suggest that lampricide sensitivity in lake sturgeon is likely strongly influenced by ontogenetic stage and physiological capacity.

Cross-endpoint integration

The combined endocrine, metabolic, and olfactory data generated in this thesis collectively indicate that yearling lake sturgeon maintained stable physiological function under the lampricide exposures tested. Cortisol and glucose data showed that the HPI axis remained fully responsive to acute handling stress, yet neither TFM nor TFM-N altered baseline endocrine state or the magnitude of the induced cortisol response at any sampling interval. Stability across acute exposure (30 minutes), prolonged exposure (12 hours), and depuration (6 days) periods indicates that lampricide exposure did not produce a detectable physiological effect capable of shifting baseline circulating cortisol or glucose or responsiveness. This pattern is

consistent with previous work by LeClair and Wilkie (2014), who demonstrated that lake sturgeon possess the capacity to biotransform TFM via glucuronidation and generate measurable quantities of TFM-glucuronide, at levels similar to those observed in rainbow trout. In that study, rainbow trout efficiently detoxified TFM at environmentally relevant concentrations and exhibited no adverse physiological effects, whereas larval sea lamprey showed minimal glucuronidation capacity and experienced pronounced metabolic disruption, including reduced hepatic glycogen. Although lake sturgeon were capable of TFM biotransformation, they were exposed to lower TFM concentrations, and the authors noted that toxic effects at higher exposures could not be ruled out. Together, these findings indicate that sensitivity to TFM in lake sturgeon cannot be attributed to a complete absence of detoxification capacity, and that physiological disturbances may occur despite measurable biotransformation, potentially reflecting constraints related to body size, energy reserves, or the effective utilization of glucuronidation pathways.

The olfactory results complement this interpretation. Blank-corrected EOG amplitudes to taurocholic acid and L-alanine were variable among individuals but did not exhibit any reduction in amplitude attributable to lampricide treatment. Because these odorants primarily stimulate ciliated and microvillous olfactory sensory neurons (OSNs), respectively, stable EOG responses suggest that receptor activation and local epithelial physiology remained intact across treatments (Hansen and Zielinski 2005; Dew et al. 2014). The concordance between endocrine and sensory endpoints is notable, given that disruptions to energy metabolism and ionic balance can influence both HPI-axis activity and olfactory transduction (Barton 2002; Sakamoto et al. 2016). Similar cross-system interactions have been reported in other teleosts

such as carp (*Cyprinus carpio*), fathead minnows (*Pimephales promelas*), and rainbow trout (*Oncorhynchus mykiss*), where contaminants such as copper or cadmium cause parallel alterations in cortisol dynamics or olfactory responsiveness, even at sublethal concentrations (De Boeck et al. 2001; Green et al. 2010; Dew et al. 2016). In YOY sturgeon, TFM exposure has been associated with reduced EOG amplitudes and altered behavioural responses to food cues (Sakamoto et al. 2016), yet no such effects were evident in yearlings in my experimental investigation. This alignment across systems indicates that the physiological processes supporting both sensory and endocrine function remained intact throughout exposure. Such stability in EOG amplitudes contrasts with studies on other fishes in which contaminants impair olfactory sensory neurons; for instance, copper typically targets ciliated OSNs while nickel selectively targets microvillous OSNs, leading to reduced EOG signals and diminished behavioural responses to food cue (Dew et al. 2014).

Within the broader context of lampricide research, this cross-endpoint stability suggests that, for yearling sturgeon under the water chemistry conditions used here, internal lampricide burden remained below thresholds required to induce detectable endocrine or sensory disturbance. The alignment of olfactory and endocrine results therefore supports the conclusion that yearlings maintain stable physiological and sensory function under TFM and TFM-N exposure, in contrast to the heightened sensitivity documented in earlier life stages.

Ontogenetic patterns and evidence from prior lampricide studies

The contrast between the present findings in yearling lake sturgeon and earlier reports of pronounced sublethal effects in YOY individuals highlights the importance of ontogeny in

shaping lampricide sensitivity. Prior laboratory and field investigations have consistently shown that smaller and younger sturgeon are more vulnerable to TFM exposure, with effects spanning olfactory, behavioural, and metabolic endpoints. For example, Boogaard et al. (2003) demonstrated that swim-up fry and fingerlings less than 100 mm in length were the most sensitive size classes, exhibiting mortality at lampricide concentrations close to operational treatment levels, particularly when exposed to TFM–niclosamide mixtures. Sakamoto et al. (2016) reported significant reductions in olfactory response amplitudes to L-alanine, taurocholic acid, and food-related cues in YOY lake sturgeon, accompanied by reduced feeding motivation, altered activity, and disrupted behavioural responses to chemosensory stimuli. In a mechanistic assessment of internal dose, Hepditch et al. (2019) showed that YOY sturgeon accumulated two to three times more TFM per gram of body mass than yearling (age 1+) fish across all water chemistries, likely due to higher mass-specific metabolic rates and greater proportional gill surface area in smaller individuals.

Tissue-level studies in larval sea lamprey, rainbow trout, and YOY lake sturgeon provide a clear mechanistic picture of how lampricides disrupt energy metabolism. In larval sea lamprey, exposure to TFM at the 12-h LC50 caused liver and brain glycogen to decline by approximately 80-85 percent, accompanied by large increases in brain lactate, substantial reductions in ATP and phosphocreatine, and no major disturbance to ion balance, indicating that toxicity stems from a mismatch between ATP supply and demand rather than failure of ionoregulation (Birceanu et al. 2009). Similar patterns have been reported in rainbow trout, where TFM exposure led to pronounced depletion of glycogen, ATP, and phosphocreatine in metabolically active tissues such as muscle, brain, and kidney, alongside elevated lactate, again

consistent with impaired oxidative ATP production and increased reliance on glycolysis (Birceanu et al. 2014). Complementary work in YOY lake sturgeon has shown that exposure to environmentally relevant TFM concentrations produces 50-80 percent reductions in ATP and glycogen in the liver and brain, elevated lactate, and a slight metabolic acidosis, confirming that cellular energy balance is highly vulnerable in this life stage (Ionescu et al. 2021). Studies with niclosamide further indicate that this compound is substantially more potent than TFM as an uncoupler of oxidative phosphorylation, causing strong depression of mitochondrial respiratory control at far lower concentrations and inducing marked metabolic disturbance in exposed fishes (Borowiec et al. 2022; Ionescu et al. 2022). Together, these findings demonstrate that lampricide toxicity in juvenile sturgeon is closely linked to disruption of oxidative ATP production and rapid depletion of energetic reserves.

The absence of detectable olfactory, endocrine, or metabolic effects in yearlings exposed to TFM or TFM-N suggests that increased body size and more mature physiological systems may provide meaningful buffering against lampricide-induced energetic strain. Hepditch et al. (2019) showed that YOY lake sturgeon had substantially higher TFM uptake rates than older fish, which the authors attributed to their higher mass-specific metabolic rates, indicating that smaller sturgeon may be more susceptible to TFM during early development. This size-dependent pattern is consistent with findings from other fish species. For example, larger juvenile black sea bream (*Acanthopagrus schlegeli*) show markedly lower mass-specific metal uptake rates compared to their smaller counterparts (Zhang and Wang 2007). Similar life-stage dependent effects have been reported in zebrafish (*Danio rerio*), where embryos exposed to TCDD (2,3,7,8 tetrachlorodibenzo-p-dioxin) exhibited markedly higher mortality, pronounced

cardiovascular toxicity, and distinct transcriptional responses compared to juveniles, despite being exposed to the same concentrations (Lanham et al. 2012). Importantly, lake sturgeon possess functional glucuronidation pathways capable of metabolizing TFM at rates comparable to rainbow trout (Le and Wilkie 2014), indicating that detoxification capacity is not a limiting factor in older juveniles under many exposure conditions. Together, these ontogenetic changes provide a potential explanation for the reduced susceptibility observed in yearlings compared to the suite of effects documented in YOY lake sturgeon (Boogaard et al. 2003; Sakamoto et al. 2016; Hepditch et al. 2019; Wilkie et al. 2019; Ionescu et al. 2021).

Environmental context interacts closely with ontogeny to influence internal lampricide burden (Hepditch et al. 2019; Wilkie et al. 2019). Under the moderate alkalinity and near-neutral pH conditions of this study, the protonation state of TFM would have favoured lower uptake across the gill relative to low-alkalinity systems, further limiting the internal dose experienced by yearlings (Bills et al. 2003; Wilkie et al. 2021). Experimental work by Hepditch et al. (2019) indicates that increased alkalinity can substantially reduce TFM uptake and associated toxicity across life stages, with stronger mitigation in older juveniles. Temperature also modulates TFM sensitivity in a non-linear manner. Bouffard (2025) reported that tolerance in 1+ lake sturgeon is highest at 8°C and 20°C and lowest at 16°C, where TFM accumulation and mortality are greatest. This pattern underscores that thermal conditions can substantially shift toxicity thresholds and interact with developmental stages to determine non-target vulnerability. Similar temperature-dependent amplification of toxicant effects has been documented in juvenile coho salmon (*Oncorhynchus kisutch*) where they experience markedly greater pesticide-induced metabolic disruption at warmer temperatures (Laetz et al. 2014). The

temperatures recorded during our exposures (approximately 15-20 °C) fall near the range in which Bouffard (2025) documented strong temperature-dependent variation in TFM tolerance, including a sensitivity peak near 16 °C.

Field evaluations of lampricide exposures provide an additional opportunity for comparison. In situ cage trials conducted during operational stream treatments have shown that age-0 lake sturgeon can experience lampricide-related mortality, with survival during treatments ranging from 45 to 100% depending on stream conditions (O'Connor et al. 2017). These field observations, together with laboratory bioassays and mitigation studies, reinforce that the youngest sturgeon represent the life stage at greatest risk, whereas larger juveniles typically exhibit higher survival under operational treatment protocols (Boogaard et al. 2003; Hepditch et al. 2019; Pratt et al. 2021). Taken together, YOY fish exhibit clear sensory, behavioural, and metabolic disturbances across a range of exposure scenarios, whereas yearlings, under moderate alkalinity and near-neutral pH, did not experience comparable sublethal effects. This integrated body of literature supports the interpretation that physiological maturity and environmental context jointly define the threshold at which lampricides transition from benign to disruptive.

Methodological considerations, limitations, and key uncertainties

The experimental design used in this thesis incorporates several methodological strengths that improve the resolution with which sublethal lampricide effects are assessed in non-target fishes. A major advantage of the framework is the integration of endocrine and sensory endpoints under exposure conditions selected to approximate operational treatments.

Combining electro-olfactography with measurements of plasma cortisol and glucose allowed simultaneous evaluation of neural and physiological endpoints, both of which have been identified as sensitive markers of lampricide exposure in young lake sturgeon and rainbow trout (Sakamoto et al. 2016; Birceanu and Wilkie 2018; Ionescu et al. 2021). By sampling across acute, prolonged, and short-term recovery periods, the study captured temporal variation in response and provided a more complete depiction of physiological trajectory than single time point assays. This approach strengthens inferences regarding the absence of measurable effects in yearlings, particularly because earlier work has shown that lampricide-induced disturbances in YOY sturgeon emerge rapidly, with pronounced physiological and sensory effects evident within the first 6 to 12 hours of exposure (Sakamoto et al. 2016; Ionescu et al. 2021).

The EOG procedures also reflect best practices for electrophysiological assessment of olfactory function in sturgeon. The use of distinct odorants that target microvillous and ciliated olfactory sensory neurons allowed evaluation of two major receptor pathways with established ecological relevance (Hansen and Zielinski 2005; Dew et al. 2014; Sakamoto et al. 2016). Consistent anaesthetic protocols, stable flow rates during odorant delivery, and the averaging of multiple replicates per fish helped minimize methodological noise and ensure that the recorded responses reflected underlying sensory physiology rather than artefactual variation. These procedures align with previous EOG studies on fish olfactory epithelia (Green et al. 2010; Dew et al. 2014).

The endocrine sampling design likewise incorporated features known to improve interpretability. Baseline sampling occurred within the recommended 3-5 minute window following netting, reducing the influence of handling artefacts (Sopinka et al. 2016; Lawrence et

al. 2018). Paired baseline and post-stressor samples allowed confirmation that the HPI axis was capable of mounting an acute cortisol response, verifying that the sampling and analytical framework had sufficient sensitivity to detect treatment-related endocrine changes had they occurred. The observed increase in cortisol and glucose after the standardized handling stressor supports this interpretation.

Despite these strengths, several limitations constrain the generality of the conclusions. First, exposures were conducted under moderate alkalinity and near-neutral pH, a chemical context known to reduce the protonated fraction of TFM and limit uptake across the gills (Bills et al. 2003; Wilkie et al. 2021). As a result, sturgeon inhabiting low-alkalinity systems may experience higher internal lampricide burdens than those tested here. In addition, water chemistry was measured prior to dosing but not continuously monitored throughout the exposures, so small fluctuations in pH or alkalinity may have occurred without being captured. Although such changes are unlikely to change the primary findings, they introduce some uncertainty regarding the exact chemical environment experienced by the fish.

Second, the experiment used a single year-class of hatchery-reared fish, which likely reduced variability in nutritional status, environmental history, pathogen exposure, and energetic condition. Wild sturgeon experience a far greater diversity of environmental pressures, including variable temperatures, flow regimes, dissolved oxygen levels, and energetic constraints (Dudgeon et al. 2006; Reid et al. 2018). These factors can influence stress responsiveness and detoxification capacity, meaning that physiological stability in hatchery-reared juveniles may represent only a subset of the responses that occur in natural systems.

Third, the physiological endpoints themselves also impose constraints on interpretation. Electro-olfactography measures responses at the level of the olfactory epithelium, but it does not assess central processing, signal integration, or behavioural outcomes. Prior work in YOY sturgeon indicates that reduced EOG amplitudes can translate into diminished attraction to food cues (Sakamoto et al. 2016), but normal EOG responses do not guarantee the absence of behavioural or ecological effects if more subtle shifts occur downstream of the epithelium. Similarly, circulating cortisol and glucose provide broad indicators of endocrine and metabolic state, but tissue-level disturbances including alterations in glycogen availability, lactate accumulation, ATP balance, or mitochondrial performance may occur without manifesting in blood-based metrics (Birceanu et al. 2009; Ionescu et al. 2021). Such subcellular effects were outside the scope of this study.

Fourth, the six-day recovery window, while sufficient to detect short-term trajectories, does not capture potential longer-term consequences of lampricide exposure. Sublethal disturbances to energy balance, immune function, or growth can arise over longer time frames, especially when organisms compensate for transient disruption by reallocating energy or modifying behaviour (Beyers et al. 1999). Because yearlings in this study showed no immediate endocrine or sensory impairment, long-term impacts are unlikely but cannot be definitively excluded.

Finally, the sample sizes, though appropriate for electrophysiological and endocrine assessments, limit the ability to detect small effect sizes. When biological variation is large, small or inconsistent treatment effects can be difficult to resolve statistically, even when sample sizes are appropriate for endocrine and electrophysiological work. For this reason, the

absence of significant differences among treatments should be interpreted in the context of underlying variability, rather than as proof that lampricides cannot produce any sublethal effects under different conditions.

Future directions

The absence of detectable sublethal effects in yearling lake sturgeon under the tested conditions highlights several avenues for future research that can refine understanding of lampricide sensitivity across life stages and environmental contexts. One important direction involves expanding assessments of olfactory and endocrine function beyond the peripheral and acute physiological endpoints measured here. Although EOG recordings provide a robust indicator of epithelial responsiveness, complementary approaches such as behavioural assays (e.g., cue-based foraging tests, odour preference trials, or assessments of exploratory behaviour) would help determine whether more subtle sensory alterations arise downstream of the olfactory epithelium. Previous work in YOY sturgeon has shown that reduced EOG amplitudes can correspond with diminished attraction to food cues (Sakamoto et al. 2016), highlighting the value of linking electrophysiological recordings with behavioural performance. Incorporating neurophysiological or central processing measures, when feasible, would further strengthen interpretations by clarifying whether lampricide exposure influences olfactory pathways beyond the level of sensory receptors.

Future studies would also benefit from examining a broader suite of metabolic indicators. While cortisol and glucose provide useful indices of acute endocrine and metabolic state, tissue-level measures such as glycogen content, high-energy phosphates (ATP and

phosphocreatine), and lactate have yielded important mechanistic insights into TFM-induced energetic disturbance in sea lamprey, rainbow trout, and juvenile lake sturgeon (Birceanu et al. 2009; Birceanu et al. 2014; Ionescu et al. 2021). Applying these approaches to yearling sturgeon may help determine whether subtle energetic or mitochondrial adjustments occur even when circulating hormones and peripheral sensory responses remain stable. This approach would be particularly informative under conditions that increase lampricide uptake, including lower pH or alkalinity environments where toxicity is elevated (Bills et al. 2003; Hepditch et al. 2019), or at temperatures that correspond with peak TFM accumulation (Bouffard 2025).

Life-stage comparisons remain a critical need. The contrast between responses documented in YOY sturgeon and the stability observed in yearlings underscores the importance of systematically evaluating lampricide susceptibility across ontogeny. A size-structured experimental framework that samples fish across a gradient of body sizes, rather than strictly by categorical life stages, would offer greater resolution for determining when susceptibility is highest and when physiological buffering capacity begins to emerge, similar to previous work done by Hepditch et al. (2019). Such information is also directly relevant to management, as it would clarify whether particular temperature-chemistry combinations create windows of increased sensitivity for specific age classes. Parallel investigations that explore how additional stressors, including elevated temperature, hypoxia, contaminants, or nutritional limitation, interact with lampricide exposure would further refine predictions for wild populations, which experience a diversity of environmental pressures absent from controlled laboratory settings.

Field-based research also represents an important next step. In situ bioassays conducted during operational TFM treatments have provided valuable data for age-0 lake sturgeon (O'Connor et al. 2017), but no comparable published field studies have focused on yearling sturgeon. Integrating controlled exposure trials with fine-scale monitoring of pH, alkalinity, flow, and lampricide concentrations would improve understanding of how fluctuating environmental parameters shape exposure risk in natural systems. Longer-term monitoring of growth, condition, and survival following lampricide exposure would further clarify whether delayed or cumulative effects emerge beyond the short recovery window examined here.

Finally, integrating physiological findings with population-level perspectives would enhance the relevance of this research for conservation and management. Physiological and behavioural responses do not occur in isolation; their ecological impact depends on how they scale to influence recruitment, growth, and long-term population trajectories. Coupling physiological endpoints with demographic modelling frameworks, such as those used to evaluate lake sturgeon recovery potential (van der Lee and Koops 2021; Vaugeois et al. 2022), would help identify whether exposure at specific life stages can meaningfully alter population dynamics under realistic environmental conditions. Such integrative approaches would support more nuanced assessments of non-target risks and help sea lamprey control programs continue balancing invasive species suppression with the conservation of native biodiversity.

Final conclusions

The results presented across this thesis contribute to an improved understanding of lampricide effects in lake sturgeon and provide a refined perspective on the balance between

invasive species control and native species protection. By demonstrating that yearling lake sturgeon experience no detectable olfactory or endocrine disruption under operationally relevant conditions, this work supports the continued use of lampricides in systems with comparable water chemistry, while underscoring the importance of life-stage-specific management approaches.

More broadly, this work highlights the value of integrating physiological endpoints into non-target risk evaluations and underscores that susceptibility to lampricides is strongly shaped by both ontogeny and environmental context. Continued integration of physiological, behavioural, and field-based assessments will strengthen the predictive capacity of non-target risk evaluations and help ensure that sea lamprey control strategies remain effective while safeguarding the long-term conservation of lake sturgeon.

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