Journal of Great Lakes Research 47 (2021) S407–S420

Contents lists available at ScienceDirect

Journal of Great Lakes Research

journal homepage: www.elsevier.com/locate/ijglr

Lampricide bioavailability and toxicity to invasive sea lamprey and non-target fishes: The importance of alkalinity, pH, and the gill microenvironment ☆

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ARTICLE INFO

Article history:
Received 31 July 2020
Accepted 28 July 2021
Available online 28 October 2021
Communicated by: Jean Adams

Keywords:
3-Trifluoromethyl-4-nitrophenol (TFM)
Acid-base regulation
Granular Bayluscide®
Lake sturgeon
Niclosamide
Respiration
Species at risk

ABSTRACT

The lampricides TFM and niclosamide are added to streams to control invasive larval sea lamprey (*Petromyzon marinus*) populations in the Laurentian Great Lakes. Lampricide effectiveness depends upon TFM and niclosamide bioavailability which is influenced by both abiotic and biotic factors. For example, at lower pH, TFM bioavailability is higher because a greater proportion exists as un-ionized TFM (TFM-OH), which easily crosses the gills. At higher pH, however, the negatively charged ionized species of TFM (TFM-O^-) predominates, which is less easily taken-up, meaning more TFM must be applied. Although water alkalinity does not directly affect TFM speciation, as a buffer it influences how much expired water crossing the gills is acidified by CO_2 and metabolic acid excretion. In poorly buffered waters, greater acidification of the expired water increases TFM bioavailability in the gill microenvironment than in better buffered, higher alkalinity waters where more TFM must be applied. Hence, sea lamprey and non-target fishes such as lake sturgeon (*Acipenser fulvescens*) are more sensitive to lampricides in low pH, low alkalinity waters. Differences in gill structure and microenvironment acidification might also explain why TFM sensitivity of young-of-the-year lake sturgeon approaches or exceeds that of sea lamprey in higher alkalinity waters. Other biotic factors such as body size and metabolic rate also contribute to differences in lampricide sensitivity. We conclude that better understanding of the abiotic and biotic factors influencing lampricide bioavailability can be used to refine treatment protocols to improve lampricide effectiveness and to better protect non-target fishes from lampricide toxicity.

Introduction

The piscicides 3-trifluoromethyl-4-nitrophenol (TFM) and 2′,5-dichloro-4′-nitrosalicylanilide (niclosamide) have been used for approximately 60 years to control invasive sea lamprey (*Petromyzon marinus*) populations in the Laurentian Great Lakes (Siefkes, 2017; Wilkie et al., 2019). Originally native to the waters and coasts of the North Atlantic Ocean, anadromous sea lamprey invaded the Great Lakes in the early 1900s following the construction of shipping canals linking the basin to the eastern seaboard of North America (see Lawrie, 1970; Smith and Tibbles, 1980; Eshenroder, 2014 for reviews). Sea lamprey spend most of their life, typically 3–7 years, burrowed in the substrate of freshwater streams as filter-feeding larvae, before undergoing a complex metamorphosis into parasitic juveniles (Youson, 2003; Manzon et al., 2015). Because the parasitic juveniles feed on the blood of large bodied fishes, the invasion exacerbated massive declines of

☆ Given their roles as Guest Editors, Mike Steeves and Michael Wilkie had no involvement in the peer-review of this article and had no access to information regarding its peer-review. Full responsibility for the editorial process for this article was delegated to Jean Adams.
This article is published as part of a supplement sponsored by the Great Lakes Fishery Commission.

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https://doi.org/10.1016/j.jglr.2021.09.005

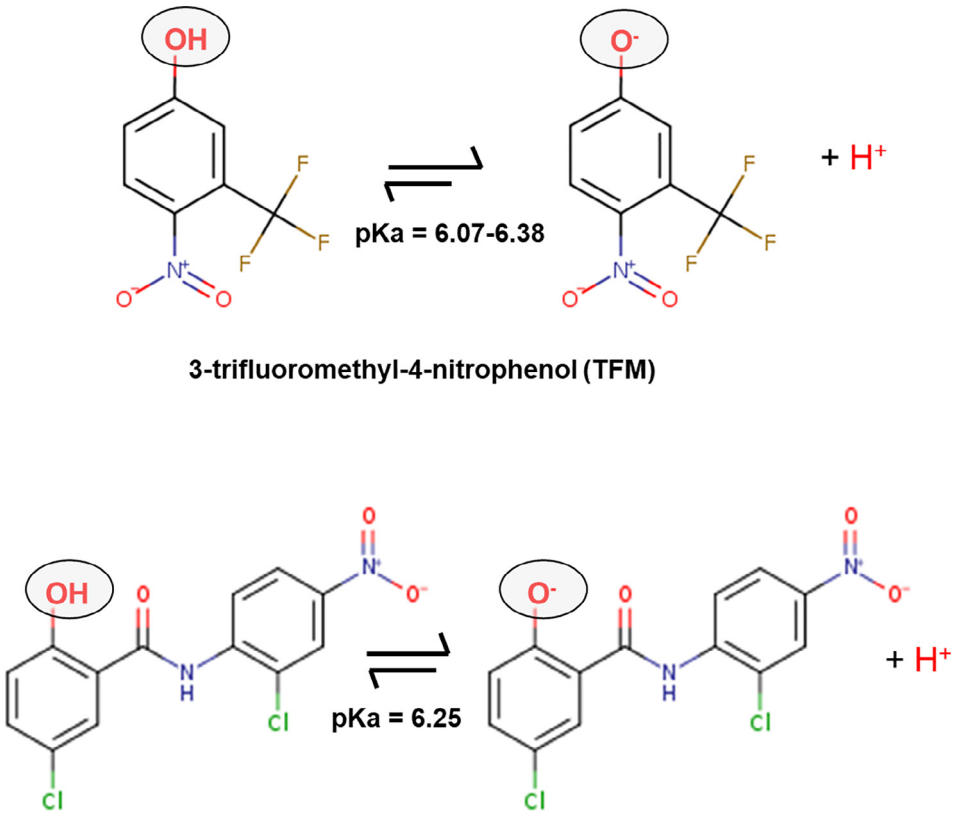

Fig. 1. TFM and niclosamide. Chemical structure and log acid dissociation constants (pKa) of the piscicides 3-trifluoromethyl-4-nitrophenol (TFM) and niclosamide (2′,5-dichloro-4′-nitrosalicylanilide) used to control invasive sea lamprey in the Laurentian Great Lakes. Chemical structures modified from https://chem.nlm.nih.gov/chemidplus.

culturally and economically important traditional, sport and commercial fish populations in the Great Lakes in the mid-20th century, including lake trout (*Salvelinus namaycush*; Smith and Tibbles, 1980; Siefkes, 2017).

Chemical control of sea lamprey populations using TFM and niclosamide (Fig. 1), more commonly called lampricides, target larval sea lamprey that live burrowed in the soft silty substrate of infested rivers and streams (Bills et al., 2003; McDonald and Kolar, 2007). Lampricides are an essential component of the sea lamprey control (SLC) program overseen by the Great Lakes Fishery Commission (Hubert, 2003), which also includes barriers to prevent the upstream migration of spawning adult sea lamprey and traps (McLaughlin et al., 2007; GLFC, 2011; Siefkes, 2017). The most widely used lampricide is TFM, which selectively targets larval sea lamprey in infested tributaries because they have a limited capacity to detoxify TFM compared to most non-target fishes (Lech and Statham, 1975; Kane et al., 1994; Bussy et al., 2018a,b). Infested rivers and streams are usually treated with TFM on a 2–4 year cycle, with mortality typically exceeding 90% (Adair and Sullivan, 2013). As a result, multiple generations of larval sea lamprey are removed with a single treatment.

Niclosamide is often used as an additive (1–2% niclosamide) with TFM, which increases TFM toxicity with little or no loss of TFM specificity to larval sea lamprey, and it reduces overall TFM use (Dawson, 2003; Wilkie et al., 2019). In addition to this emulsifiable formulation of niclosamide, a bottom-acting formulation of niclosamide called granular Bayluscide® (gB) is used in large rivers and lentic habitat where TFM would be diluted and impractical due to high rates of water discharge or depth (Fodale et al., 2003; Scholefield et al., 2003). Unlike TFM, which is restricted to sea lamprey control, niclosamide is also a molluscicide that is applied to waters infested with snails that are vectors for the parasitic worm that causes schistosomiasis in humans, and as an anthelmintic to treat intestinal cestode (tapeworm) and fluke infections in mammals (Lardans and Dissous, 1998; Köhler, 2001; Zhao et al., 2015).

In both sea lamprey and non-target fishes, TFM and niclosamide are thought to cause toxicity by uncoupling mitochondrial oxidative phosphorylation (Niblett and Ballantyne, 1976; Birceanu et al., 2011; Huerta et al., 2020), the oxygen dependent (aerobic) process used by most organisms to generate ATP, the main energy currency used to power life-dependent biological processes (Birceanu et al., 2009; Clifford et al., 2012). This forces the animals to rely on anaerobic (non-oxygen dependent) energy reserves such as glycogen and phosphocreatine, to make-up for shortfalls in aerobic ATP production. These anaerobic stores are finite, however, and death ensues when ATP demands can no longer be met (Wilkie et al., 2007; Birceanu et al., 2009; Clifford et al., 2012). Notably, sea lamprey, rainbow trout (*Oncorhynchus mykiss*) and lake sturgeon (*Acipenser fulvescens*) that survive treatment readily restore their internal energy stores, and appear not to suffer from any lingering effects of lampricide exposure (Clifford et al., 2012; Ionescu et al., 2021).

In the case of TFM or TFM plus niclosadamide mixtures, lampricide effectiveness can be reduced by dilution arising from heavy rain events, the presence of groundwater upwellings, seeps and backwaters that dilute lampricide, and low rates of water discharge and even beaver dams, which can also interfere with lampricide delivery (reviewed by Sullivan et al., 2021). These factors can

increase the risk of residual larval sea lamprey that survive treatment, but can be minimized through reconnaissance, preparation, and advance planning prior to treatments (Christie et al., 2003; Adair and Sullivan, 2013). However, there are other factors that treatment crews have little control over such as the sea lamprey's behaviour, physiology, and environment. The benefits of behaviours such as avoidance and escape from lampricide exposure are self-evident, but understanding how physiological and environmental variables influence the sensitivity of sea lamprey, not to mention non-target organisms to lampricides, is much more complicated.

The following synthesis arose from the 3rd Sea Lamprey International Symposium (SLIS III) hosted by the Great Lakes Fishery Commission (GLFC) in Detroit, Michigan, which brought together many of the world's lamprey researchers and biologists to exchange knowledge and ideas related to sea lamprey control and the conservation of native lampreys around the world. The purpose of this paper is to explore how key biotic and abiotic factors influence the sensitivity of sea lamprey and non-target organisms to lampricides by affecting lampricide bioavailability, which is defined as the extent to which a substance is free to be taken up by an organism and have a biological effect (Newman and Unger, 2003). Beginning with a review of the current model of lampricide toxicity, we then discuss how two key abiotic factors, water pH and alkalinity, affect the TFM sensitivity of sea lamprey and the mechanisms that underpin their responses to lampricides. This is followed by a discussion of how biotic variables such as life stage and body size, and physiological processes such as gas exchange and acid-base regulation at the gills interact with abiotic (pH, alkalinity) variables to influence lampricide effectiveness. This paper will mainly focus on TFM, which is used for most lampricide treatments. Its modes of action in sea lamprey and fishes are better understood than niclosamide, at least at this point in time. We acknowledge that neither the abiotic and biotic features that affect TFM sensitivity operate in complete isolation, rather they tend to be interactive in nature as will be evident throughout this article.

We will begin by introducing the current model of TFM toxicity, with inferences to the mode(s) of niclosamide action. This will be followed by a discussion of what is known about how key abiotic factors such as water pH and alkalinity affect the TFM sensitivity of sea lamprey, before focusing on the related mechanisms that underpin the responses of sea lamprey to lampricides. Particular attention will be given to how biotic variables such as body size and physiological processes related to gas exchange and acid-base regulation taking place at the gill surface interact with these abiotic variables.

Given ongoing concerns about the potential effects of lampricides on non-target vertebrate and invertebrate species, we will also address how these abiotic and biotic features affect lampricide sensitivity in the lake sturgeon, a species at risk in some jurisdictions (Golder Associates Ltd., 2011; COSEWIC, 2017). Lake sturgeon were once abundant in the Great Lakes-St. Lawrence region, but populations there and elsewhere were devastated by overharvest, fragmentation due to dams and navigation locks, and habitat degradation (Harkness and Dymond, 1961). Efforts to restore their populations have been moderately successful, particularly in the Lake Huron-Lake Erie corridor (Pollock et al., 2015; Welsh et al., 2017). There are concerns, however, about the potential effects that lampricides could have on lake sturgeon conservation and restoration initiatives (Dobiesz et al., 2018; reviewed by Pratt et al., 2021).

Current model of lampricide toxicity

Our present understanding of the mechanisms underlying TFM and niclosamide toxicity were recently described in detail by Wilkie et al. (2019) and Birceanu et al. (2021). Both TFM and niclosamide interfere with aerobic ATP production in the mitochondria. Aerobic ATP production takes place via oxidative phosphorylation, a pathway that combines oxidative reactions in the electron transport chain (ETC) of the mitochondria with the phosphorylation of ADP, to generate ATP, the body's primary energy currency. When the body's energy stores (carbohydrate, lipid, protein) are metabolized they enter the citric acid cycle within the mitochondria, which ultimately leads to the generation of the energy substrates $FADH_2$ and NADH, which then enter the ETC resulting in the consumption of O_2, and the generation of water, heat, and reactive oxygen species. This process is also used by the ETC to generate an electrical (potential) and H^+ (ΔpH) gradient between the intermembrane space of the mitochondrial matrix, the proton motive force ($\Delta\Psi$). As a result of the $\Delta\Psi$, there is a large electrochemical gradient that favours the movement of H^+ from the intermembrane space into the matrix, which is limited due to the relatively low H^+ permeability of the inner membrane. When ATP demands increase, H^+ flows (downhill) through the ATP synthase, also called the F_0F_1-ATPase, which releases the energy needed to phosphorylate ADP to ATP (see Moyes and Schulte, 2008 for review). There is strong evidence that TFM "uncouples" this process by interfering with the generation of the $\Delta\Psi$, which decreases the electrochemical gradient that favours the H^+ passage through the ATP synthase, lowering ATP production (Niblett and Ballantyne, 1976; Birceanu et al., 2011; Huerta et al., 2020). Based on studies in mammalian systems, niclosamide appears to work in a similar manner to TFM (Van den Bossche, 1985; Park et al., 2011; Alasadi et al., 2018) but its precise mechanism in fishes has not yet been determined. One means by which both TFM and niclosamide are thought to uncouple oxidative phosphorylation is by acting as "proton shuttles" that transport H^+ from the intermembrane space to the matrix, which lowers the $\Delta\Psi$ and therefore lowers ATP production in a dose-dependent manner (Birceanu et al., 2011; Park et al., 2011; Jurgeit et al., 2012; Huerta et al., 2020).

A consequence of impaired ATP production by lampricides for both sea lamprey and non-target fishes is that it leads to the rapid depletion of anaerobic sources of ATP including high energy phosphagens such as phosphocreatine (PCr) in fishes and phosphoarginine in molluscs (Birceanu et al., 2009, 2014; Clifford et al., 2012; Henry et al., 2015; Viant et al., 2001; Wilkie et al., 2007). There is also a greater reliance on anaerobic glycolysis, which leads to marked reductions in tissue glycogen stores and the accumulation of lactate along with acid-base disturbances (Wilkie et al., 2007; Birceanu et al., 2009, 2014; Clifford et al., 2012; Henry et al., 2015; Ionescu et al., 2021). When these finite, anaerobic sources of ATP are too low, ATP supply can no longer meet ATP demand, which results in homeostatic disturbances and death soon after.

Effects of pH on TFM and niclosamide sensitivity

Originally, TFM treatment concentrations were based on water alkalinity and conductivity (Seelye et al., 1988). However, it was evident early in the SLC program that the effectiveness of TFM was influenced by water pH (Applegate et al., 1961). Today, the importance of water pH is reflected by the constant, careful monitoring of water pH that takes place during treatments and the essential role that it plays in determining the TFM target concentrations used during lampricide applications (Bills et al., 2003; Barber and Steeves, 2019). In both sea lamprey and non-target fishes, TFM toxicity decreases as water pH increases (Fig. 2; Bills et al., 2003; McDonald and Kolar, 2007; Wilkie et al., 2019). The rates of TFM application during a lampricide application are based

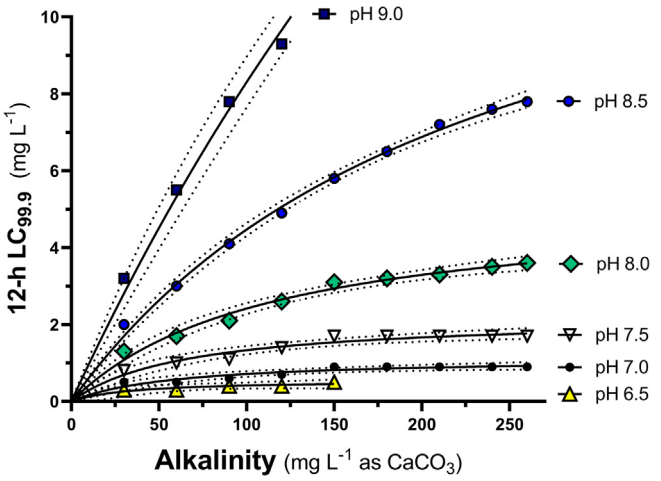

Fig. 2. Relation between TFM toxicity, pH and water alkalinity in larval sea lamprey. Toxicity is expressed as the 12-h minimum lethal concentration (MLC) of TFM to sea lamprey, which is equal to the 12-h $LC_{99.9}$. Data were fitted by standard non-linear regression using the least squares fitting method (GraphPad Prism 8, San Diego, CA). Dotted lines (···) denote 95% confidence intervals. Data were re-plotted from Bills et al. (2003).

on the minimal lethal concentration (MLC) of TFM over a 9-h exposure, which is equivalent to the concentration of lampricide needed to kill 99.9% of the population of sea lamprey over 9 h (9-h $LC_{99.9}$) at a given pH and alkalinity (Bills et al., 2003). The MLC is sometimes determined using streamside toxicity tests at treatment sites, but more commonly from treatment history and from tables in which the target MLC is based on laboratory bioassays conducted at different combinations of pH and alkalinity (Bills et al., 2003). The underlying goal is to ensure that all sea lamprey in the treated system are exposed to the MLC or higher for a minimum of 9 h, which usually necessitates adding TFM to the system at 1.3–1.5 times the MLC over approximately 12–16 h (Bills et al., 2003; McDonald and Kolar, 2007) to account for attenuation and dilution of the TFM as it moves downstream. Some sources of dilution include exchange of TFM-laden water with the underlying hyporheic zone of the river, ground water upwellings, inflow from downstream tributaries or downstream variation in water chemistry (Barber and Steeves, 2019).

Water pH determines the bioavailability of TFM and niclosamide to sea lamprey and non-target fishes (Hunn and Allen, 1974; McDonald and Kolar, 2007; Wilkie et al., 2019). Bioavailablity is influenced by numerous variables in aquatic environments including the free concentration of a substance, which is influenced by the total dissolved concentration of the substance, its binding to suspended sediments or stream substrate (McConville et al., 2017a, b), and its chemical speciation, which in turn influences how easily a substance can be taken up (Hunn and Allen, 1974; Hlina et al., 2017). Both TFM and niclosamide are weak acids, each containing ionizable OH functional groups, that give up their H^+ as water pH increases. As a result, they are present as either the more lipid soluble (more lipophilic), un-ionized form (TFM-OH) that is readily taken up across the epithelial tissue comprising the gill, or the less lipid soluble (more hydrophilic) ionized form (TFM-O-; Fig. 1).

The degree to which TFM and niclosamide dissociate from their un-ionized to ionized states is determined by the pKa (the negative log of the acid dissociation constant, Ka, for an acid-base reaction), which can be used to calculate the relative concentrations of the un-ionized versus ionized form of each agent using the Henderson-Hasselbalch equation (McDonald and Kolar, 2007). More simply, the pKa is the pH where 50% of a weak acid is in its un-ionized form and 50% is in its ionized form. The respective pKa's, between 6.07 and 6.38 for TFM (Hubert, 2003; McConville et al., 2016) and 6.25 for niclosamide (Dawson, 2003), indicates that at lower pHs, greater than 50% of TFM and niclosamide exists in their un-ionized form (TFM-OH, Nic-OH), but as water pH increases the ionized forms (TFM-O^- and Nic-O^-) predominate (McDonald and Kolar, 2007; Fig. 3A). In practical terms, this means that the majority of TFM and niclosamide would be in the less bioavailable, ionized form at the circumneutral water pHs that are typical of most waters draining into the Great Lakes (~pH 7.0–8.5).

It should be kept in mind that because pH expresses the concentration of H^+ on a logarithmic scale, the effect of a 1 unit change in water pH would represent a 10-fold difference in H^+ concentration, with corresponding changes in the concentration of the more bioavailable, un-ionized TFM or niclosamide. Indeed, the accumulation of radiolabelled TFM (^{3}H-TFM) by rainbow trout over 6 h was more than 20-fold higher in fish exposed to the lampricide in water at pH 6.0 compared to pH 9.0 (Hunn and Allen, 1974).

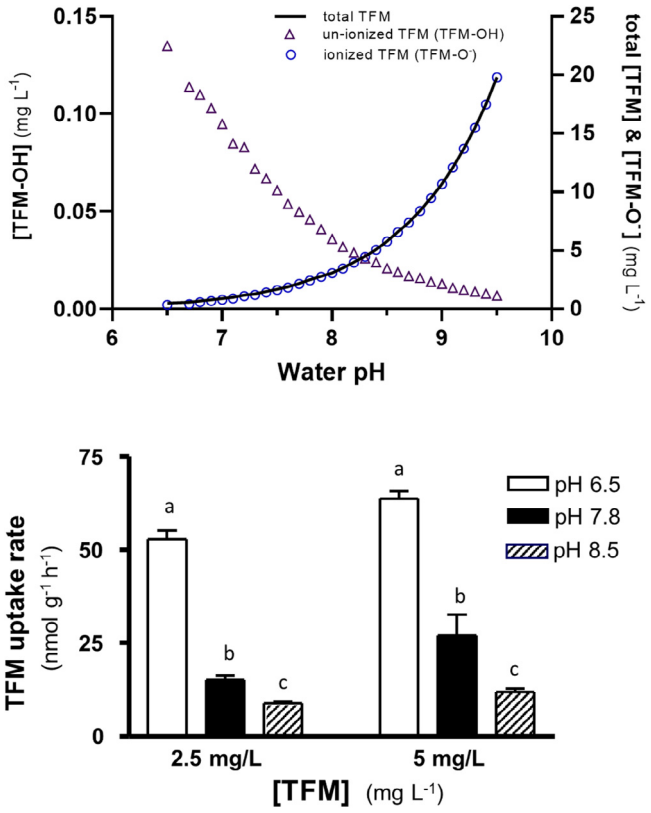

Fig. 3. The effect of water pH on TFM speciation and rates of TFM uptake by larval sea lamprey. (A) Speciation of TFM calculated by re-arrangement of the Henderson-Hasselbalch equation where pH = pKa + log ([TFM-O^-]/[TFM-OH]), where [TFM-O^-] and [TFM-OH] are the concentrations of ionized TFM (open circles) and un-ionized TFM (triangles), respectively (McDonald and Kolar, 2007). Note that as the water pH increases, the proportion of bioavailable TFM-OH decreases at a given total TFM concentration (solid line; total TFM = TFM-OH + TFM-OH^-). Data were calculated from published values of the expected minimum lethal concentrations (MLC = 12-h $LC_{99.9}$) of total TFM at different water pHs, and a constant water alkalinity of 150 mg L^{-1} as $CaCO_3$ (Bills et al., 2003). (B) Rates of TFM uptake were measured using radio-labelled ^{14}C-TFM, which was added to the water along with non-radiolabeled TFM. The TFM uptake rate was equal to the amount of ^{14}C-TFM absorbed (based on beta radiation measurements) per gram whole body mass, divided by the mean specific activity of ^{14}C-TFM (counts per minute ^{14}C-TFM $nmol^{-1}$ non-radiolabeled TFM) and time of exposure to TFM (1 h). Mean TFM uptake rates (±1 standard error) with different lowercase letters are significantly different from one another at each exposure concentration ($P < 0.05$) as determined by analysis of variance (ANOVA) followed by Tukey's post-test. From Wilkie et al. (2019).

Using ^{14}C-TFM, it was demonstrated in sea lamprey that TFM uptake decreased in a step-wise fashion as water pH was increased, in accord with corresponding reductions in the concentrations of un-ionized TFM in the water (Fig. 3B; Hlina et al., 2017; Wilkie et al., 2019).

The effects of water pH on niclosamide are less studied, and this is likely related to the fact that niclosamide (1–2%) is often used to enhance TFM toxicity without substantial loss of TFM specificity to sea lamprey. Consequently, the primary focus would be pH effects on TFM. Water pH appears to have similar effects on niclosamide sensitivity of sea lamprey, but to a much lesser extent than TFM. For instance, the 12-h LC_{99} of niclosamide in larval sea lamprey increased as water pH increased from pH 6.5 to pH 8.5 (Fig. 4A; Dawson et al., 1977). Similar increases in water pH from pH 6.5 to pH 9.5 resulted in a steady increase in the niclosamide 96-h LC_{50} (concentration required to kill 50% of the population over 96 h) of rainbow trout (Fig. 4B; Bills and Marking, 1976).

The implications of pH on niclosamide bioavailability could be relevant for improved use of the granular formulation of niclosamide (gB), which is used to treat larger, deeper river systems and lentic habitat (Dawson, 2003; Scholefield et al., 2003). Indeed, the pH of interstitial water (pore water) to which the larval sea lamprey, not to mention various species of native lampreys, mussels, and other invertebrates, are exposed in the bottom substrate can vary substantially from the overlying water column. For instance, pH was on average about 0.4–0.5 pH units lower in pore water (measured pH ~ 7.4–7.6) than in samples collected from the overlying water column in the upper Mississippi River (~pH 8.0; Frazier et al., 1996). Unlike TFM or TFM plus niclosamide (1–2%) mixture treatments, which are based on the MLC of TFM over several hours, bottom-acting gB is applied on mass per unit area basis and intended to kill sea lamprey in a matter of minutes before the chemical is diluted by water currents (Scholefield et al., 2003). As a result, far less is known about the concentration, let alone the bioavailability of niclosamide to sea lamprey and non-target organisms occupying the interstitial water. Thus, a better knowledge of how the speciation of niclosamide is influenced by pH, and how this affects the rates of uptake, distribution, and elimination of niclosamide, could lead to improved predictions of gB treatment effectiveness and its potential non-target effects.

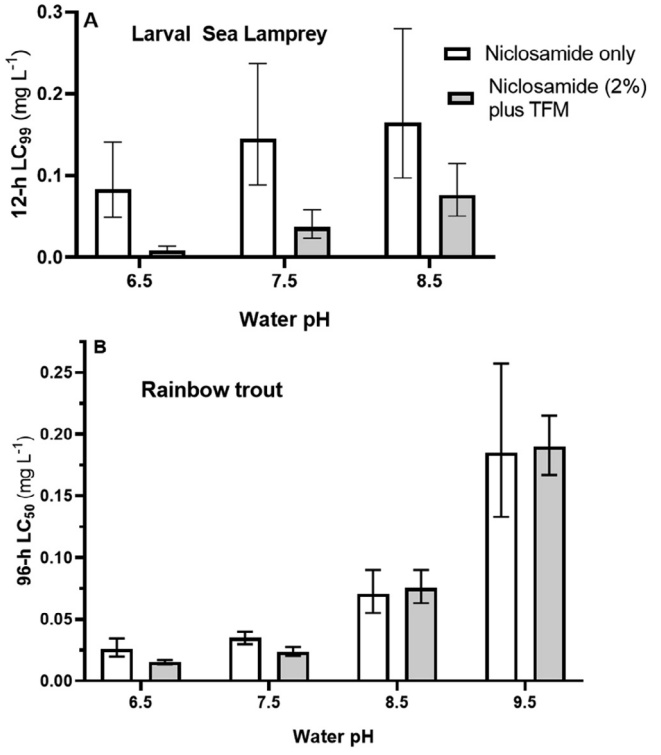

Fig. 4. Comparison between niclosamide toxicity and water pH. Measurements of (A) the 12-h $LC_{99.9}$ [±95% confidence interval (CI)] of niclosamide in sea lamprey or (B) the 96-h LC_{50} (±95% CI) of rainbow trout exposed to niclosamide only (open bars) or to a niclosamide-TFM mixture (2% niclosamide: 98% TFM; solid bar) at different water pHs. All measurements were made in soft water (hardness = 44 mg L^{-1} as $CaCO_3$) at 12 °C. Data from Bills and Marking (1976) and Dawson et al. (1977).

Another question is how pH affects toxicity when TFM and niclosamide are used in mixtures, but to resolve this question more needs to be known about the nature of their interactions, which can either be additive, less than additive (antagonistic), or greater than additive (synergistic). An additive interaction occurs when the observed toxicity or effect is equal to the sum of the expected effects for each toxicant, whereas a synergistic effect results when the observed toxicity is greater than the combined effect, and an antagonistic effect is when the response is less than the expected (Newman and Unger, 2003). Dawson (2003) reported that the TFM and niclosamide acted together in an additive manner, but recent work indicates that in rainbow trout the interactions are in fact synergistic when used at environmentally relevant concentrations (Hepditch et al., 2021). Regardless, an unresolved question would be whether or not the bioavailability of TFM and niclosamide change in proportion to one another with water pH, which could alter the relative amounts of each agent to which sea lamprey and non-target organisms are exposed. A cursory comparison of the comprehensive dataset relating TFM toxicity to pH and alkalinity (Fig. 3; Bills et al., 2003) to that relating niclosamide toxicity to pH (Fig. 4; Bills and Marking, 1976; Dawson et al., 1977), indicates a weaker relation between niclosamide and pH. Nevertheless, a better understanding of the acid-base chemistry, as well as the other chemical properties of niclosamide is needed to help guard against residual larval sea lamprey or mortality of non-target organisms.

Effects of water alkalinity on TFM and niclosamide sensitivity

As with water pH, it has long been recognized that alkalinity markedly influences the sensitivity of sea lamprey and non-target fishes to TFM (Kanayama, 1963; Seelye et al., 1988; Bills et al., 2003). The U.S. Geological Survey (https://www.usgs.gov/special-topic/water-science-school/science/alkalinity-and-water) defines alkalinity as "the ability of a water body to neutralize acids and bases and thus maintain a fairly stable pH level". This definition is useful because it recognizes the capacity of waters to "buffer" or neutralize changes in water pH when acid (i.e., H^+) or base (i.e., OH^-) is added to the system. In most freshwater ecosystems, the two most important buffers are bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}), which neutralize acid (H^+) that is added to the system and therefore allow well buffered waters to maintain a relatively stable pH.

Typically, alkalinity is measured by titration, in which known amounts of strong acid (e.g., HCl, HNO_3) are incrementally added to the water until reaching a pre-determined water pH (e.g., pH 4.0) where all the buffer (HCO_3^-, CO_3^{2-}) is consumed by the reaction. For this reason, terms such as titratable alkalinity, titratable base, carbonate alkalinity, or alkaline reserve are considered synonymous with the term alkalinity (Wetzel, 1983). Typically, alkalinity is expressed in mg L^{-1} as $CaCO_3$, which refers to the amount of H^+ needed to consume all of the carbonates (HCO_3^-, CO_3^{2-}) present in the water, with values directly proportional to buffer capacity. In the Great Lakes basin, alkalinity ranges from lows of <25 mg L^{-1} as $CaCO_3$ in poorly buffered waters of tributaries underlain by

granite bedrock to the highly buffered waters of tributaries underlain by limestone ($CaCO_3$) with alkalinities near 250 mg L^{-1} as $CaCO_3$ (Sullivan et al., 2021).

Because it was recognized that alkalinity was related to TFM sensitivity (Kanayama, 1963; Seelye et al., 1988), alkalinity, along with conductivity, was used to calculate the TFM concentrations required for lampricide treatments in the early years of the SLC program (Bills et al., 2003). It was only later, following the ground-breaking work of Bills et al. (2003), when pH was factored into lampricide toxicity models, leading to more accurate determination of TFM treatment concentrations and ultimately reduced TFM consumption, treatment frequency, and non-target effects (Sullivan et al., 2021). A strength of the model is that it implicitly recognizes the inter-relation between alkalinity and pH, and how pH directly affects TFM speciation. However, the underlying mechanisms for the relation between alkalinity and TFM toxicity remain poorly understood. Unlike pH, alkalinity does not directly alter the speciation of ionizable compounds. However, by determining water buffer capacity, water alkalinity could indirectly alter the speciation of TFM by modulating the pH of water at the surface of the gills, the site of TFM uptake, as proposed below.

Gill function and lampricide toxicity

The gills are a multifunctional organ responsible for gas exchange, nitrogenous waste excretion (ammonia, urea), ion exchange, and acid-base regulation (Wilkie, 2002; Wilson and Laurent, 2002; Evans et al., 2005). There are some obvious structural differences in the fundamental organization of teleost fish gills (Fig. 5A–C) and larval lamprey gills (Fig. 5D–F), such as the presence of seven gill pores (branchiopores) in lampreys, which open into branchial pouches in which the gills are supported by a cartilaginous lattice (branchial basket), rather than the gill arches and external gill cover (operculum) present in teleosts (Randall, 1972; Hughes, 1984; Wilson and Laurent, 2002). The functional units of the gills, however, are very similar comprising gill filaments (also called primary lamellae) containing numerous plate-like lamellae (secondary lamellae), which are the primary gas exchange site (Fig. 5C, F; Hughes, 1984; Lewis, 1980). Blood flows through the gills via afferent and efferent arterioles, which regulate blood flow through lamellar capillaries, the sites of red blood cell O_2 uptake and CO_2 excretion. In both lampreys and fishes, blood flow is countercurrent to water flow, with each passing in the opposite direction, allowing the lamellae to maximize gas exchange efficiency. The filamental and lamellar epithelium also contains different types of mitochondria rich cells (MRCs), ammocoete MRCs and intercalated MRCs in larval lampreys (see Bartels and Potter, 2004; Zydlewski and Wilkie, 2013 for reviews), and α and β MRCs in teleosts (see Bartels and Potter, 2004; Dymowska et al., 2012; Ferreira Martins et al., 2021 for reviews). A detailed description of MRCs is beyond the scope of this article, but they are thought to play a key role in regulating internal electrolyte and acid-base balance through the uptake of ions such as Na^+ and Cl^- from the water, and the exchange of acid (H^+) and base (HCO_3^-) equivalents with the water, respectively. Readers are

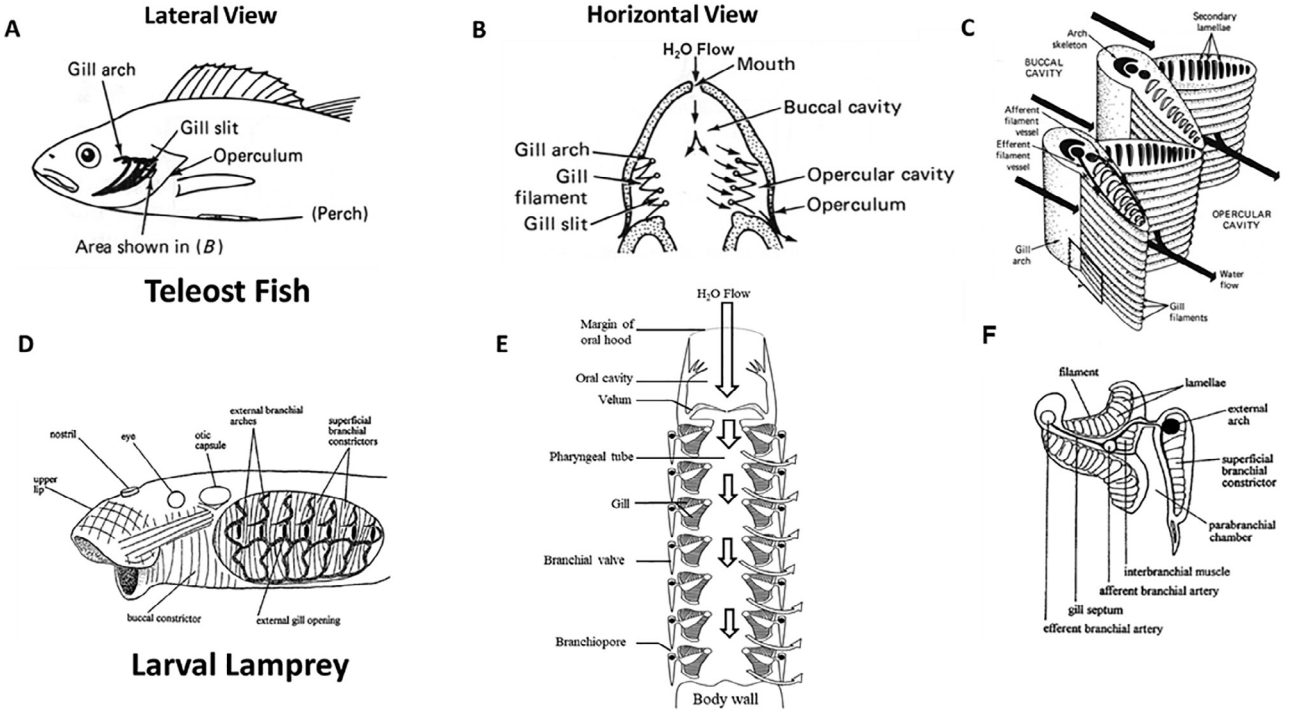

Fig. 5. Gill structure and function in teleosts and lampreys. Lateral views of the general organization of the gills in teleosts and lampreys. Note the presence of four gill arches and five gill slits (A) which separate the buccal cavity from the opercular cavity, and the presence of a gill cover (operculum) in the teleost fish (A, B). Irrigation of the gills takes place using a buccal-opercular pump (B), in which water is drawn into the mouth and across the gills following expansion of the buccal cavity volume and opercular cavity due to a lowering of the buccal cavity floor and outward flexion of the operculum, respectively. A subsequent increase in buccal cavity pressure due to elastic recoil further maintains water flow across the gills. In the lamprey the seven gill openings (branchiopores; D) are irrigated using a "velar pump" comprising a muscular velum (F), which separates the oral cavity from the pharyngeal tube. Contraction of the velum increases the volume of the oral cavity, allowing water to enter via the oral hood, followed by rising water pressure that enters the pharyngeal tube when the velar muscles and pharyngeal muscles relax, expanding the volume of the pharyngeal tube, which draws in water down its pressure gradient. Increases in water pressure due to contraction of the velum and contraction of the pharyngeal musculature force water across the gills, accompanied by contraction of brachial constrictor muscles that increases the volume of the parabranchial chamber to facilitate water flow across the gills when the pharyngeal muscles relax (E, F). The functional units of teleost and lamprey gills are the plate-like gill lamellae (secondary lamellae; C, F), which are distributed along the gill filaments. Water passes between the lamellae of the gills as the gills are irrigated, in a direction that is countercurrent to capillary blood through the lamellae maximizing O_2 uptake and CO_2 excretion. Illustrations modified from Mallatt (1996), Hill and Wyse (1989), and Wilkie (2011).

referred to the up-to-date review by Ferreira-Martins et al. (2021) on this topic.

The excretion of H^+ by the gills tends to make the water at the gill surface more acidic as the water moves from the leading to trailing edges of the lamellae (Playle and Wood, 1989; Erickson et al., 2006), as does CO_2 excretion, which is hydrated to HCO_3^- and H^+ within gill epithelia cells, as well as at the gill surface (Wright et al., 1986; Playle and Wood, 1989). By making the gill microenvironment more acidic than the bulk water, a greater proportion of TFM is in its bioavailable, phenolic (TFM-OH) form at the gill surface compared to the bulk water. However, the degree of acidification taking place in the gill microenvironment is inversely proportional to the alkalinity of the water. In other words, as the buffer capacity increases at higher alkalinities, the amount of acidification at the gill surface is expected to decrease, as we recently demonstrated using rainbow trout fitted with surgically implanted opercular catheters that allowed us to measure the pH of the expired gill water (Fig. 6A). At low alkalinity (and bulk water pH greater than 6.5), the degree of expired gill water acidification observed was much higher compared to moderate, or high alkalinity, where virtually no acidification of the expired water was observed (Fig. 6B). Thus, in a fish exposed to TFM, the difference in TFM-OH concentrations would be greatest in waters of lower alkalinity (e.g., low buffer capacity), where acidification at the gill

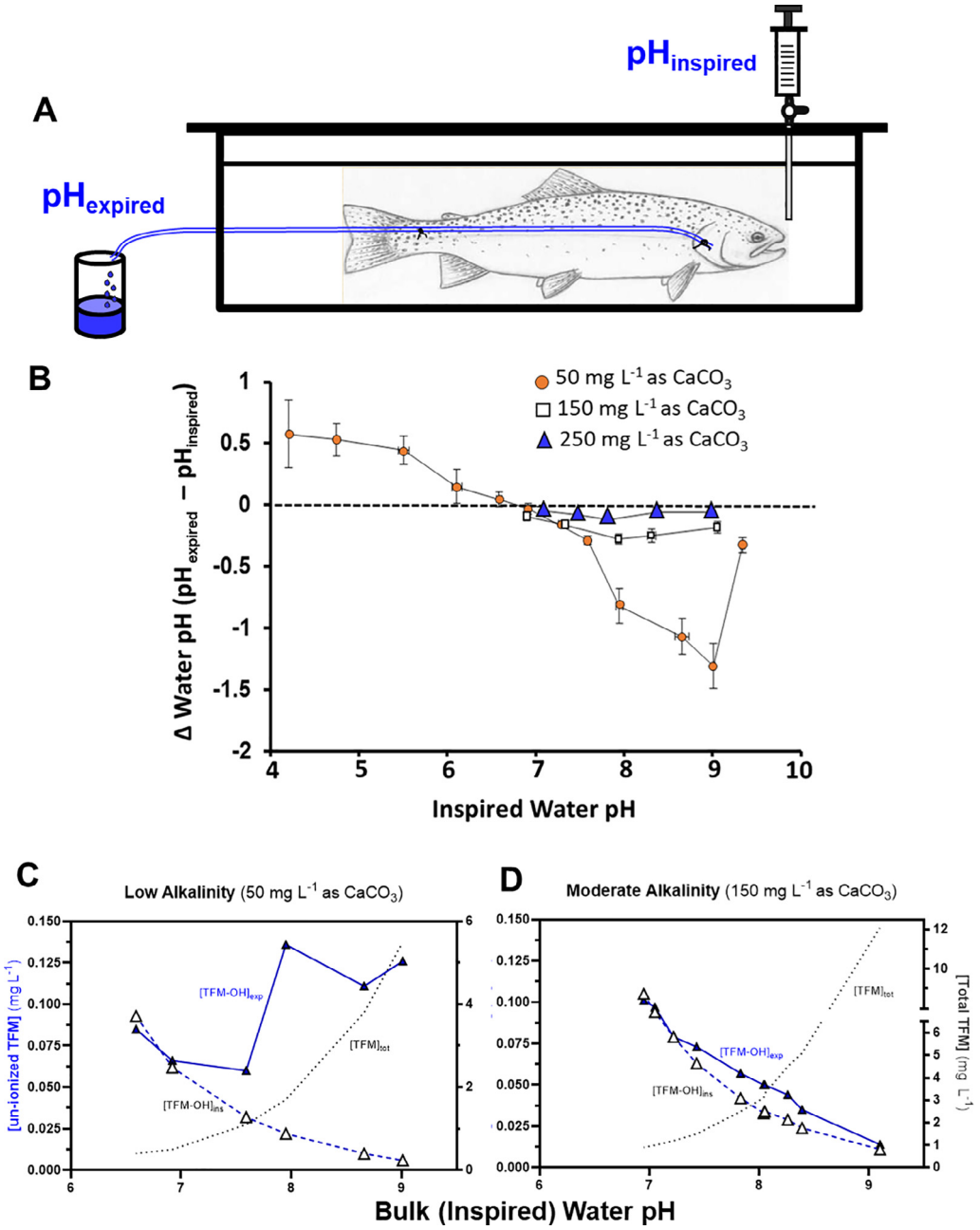

Fig. 6. Effects of the gill microenvironment on TFM speciation. (A) Rainbow trout fitted with an opercular catheter, used to draw expired water from the opercular chamber of the gill for measurement of water pH at the surface of the gill. (B) Relation between expired (gill surface) pH of rainbow trout acclimated to different water pHs and alkalinities for a minimum of 2 weeks. Note that in fish acclimated to low alkalinity water (50 mg L^{-1} as $CaCO_3$), there was net alkalinization (increased pH) of the expired water when the inspired (bulk water) pH was <7.0. Above pH 7.0, there was net acidification of the expired water. (C, D) Relation between the concentration of total TFM (dotted line), the proportion of un-ionized TFM (TFM-OH) at the gill surface (expired water; solid line), and inspired (bulk; dashed line) water in fish acclimated to water of lower (C) compared to higher (D) alkalinity.

surface would be greatest, decreasing as alkalinity increased, until there were no differences at alkalinities above 200 mg L^{-1} as $CaCO_3$. This is illustrated by the following analysis using rainbow trout expired pH measurements, which can be considered representative of water pH at the gill surface. As demonstrated in Fig. 6B, in waters of low alkalinity and low pH (<6.5) there was a net addition of base to the water, leading to an increase in water pH compared to the inspired (bulk) water. Similar findings have been reported in previous studies, using similar approaches and are thought to be due to NH_3 excretion (Playle and Wood, 1989). Nevertheless, few if any streams containing larval sea lamprey would be in this pH range. However, between pH 6.5 and 9.0, waters that are known to be habitable for sea lamprey, the difference between the expired water pH (gill surface) and inspired water pH (bulk water) becomes more negative as the expired water becomes more acidic (Fig. 6B). The consequence for fish living in waters of low alkalinity and exposed to TFM is that the bioavailability of TFM would be greater at the gill surface than if it were predicted based on measures of the bulk water only. This is made in clear in Fig. 6C, in which the bioavailable, un-ionized TFM (TFM-OH) is several-fold higher at the gill surface, than it is in the inspired (bulk) water. However, in better buffered waters of moderate to high alkalinity, the degree of acidification at the gill surface is much less, as revealed by the markedly lower difference between the un-ionized TFM in the inspired and expired gill water (Fig. 6D).

While the above model explains how acidification at the gill surface likely alters TFM bioavailability in teleost fishes, it is likely applicable to lampreys as well. Unlike teleost fishes, which use a buccal-opercular pump to irrigate the gills (Fig. 5B; Hughes, 1984), larval lampreys generate respiratory currents across the gills using the muscular velum (velar flaps) anchored to the anterior portion of the branchial basket (Fig. 5E; see Rovainen, 1996; Wilkie, 2011 for reviews). During inspiration, the flaps seal together and contract in a caudal direction, which expands the volume and decreases the pressure in the oral cavity, drawing in water via the oral hood. Contraction of the branchial muscles surrounding the pharynx, squeezes water in the pharynx across the gills, which then exits via the branchiopores. Relaxation of the velum and the branchial muscles leads to an elastic recoil of the pharyngeal chamber which draws in water from the oral cavity, and then the cycle repeats (Rovainen, 1996). As in teleost fishes, this results in (near) continual unidirectional water flow across the gills. Because the gills are internalized within the branchiopores, similar acidification of water in the gill microenvironment due to CO_2 excretion would be expected in larval lampreys, as it is teleosts. Plus, the gill epithelium of lampreys, like teleosts, also has H^+ excreting V-ATPases (Bartels and Potter, 2004; Reis-Santos et al., 2008; Sunga et al., 2020), which would also contribute to acidification of the gill water. Whether or not there are quantitative differences in the amount of acidification between lampreys and teleosts remains to be determined, but it is worth noting that larval sea lamprey excrete metabolic acid at very high rates following exhaustive exercise (Wilkie et al., 2001). Future studies on sea lamprey that are directed at quantifying how gill microenvironment pH differs from the bulk water pH at different alkalinities would make it possible to make better predictions about how alkalinity influences actual TFM bioavailability, as well as niclosamide bioavailability.

Other possible routes of lampricide uptake

The above analysis assumes that TFM is taken up via the gill, rather than via the skin or by ingestion. The suggestion that some TFM would be taken up via the skin is supported by observations that it may be a site of gas exchange based on the presence of dermal capillaries in the skin of adult sea lamprey, and the larvae of a few other lamprey species (Potter et al., 1995). Conspicuously, however, dermal capillaries near the skin surface were not observed in larval sea lamprey (Potter et al., 1995), which makes it less likely that TFM would be taken up across the skin. Another consideration is that the much lower relative surface area of the skin compared to the extremely well-vascularized gill, makes it less likely that there would be substantial lampricide uptake via the skin. Also, there would be relatively little convective flow of water across the skin of a larval sea lamprey to deliver lampricide, whereas the velar-pump of the larvae continually irrigates the gills with oxygen and food-particle laden water (Lewis, 1980; Rovainen, 1996; Mallatt, 1996) providing an effective lampricide delivery system.

If there were an accessory route of TFM uptake, ingestion of TFM via the gastrointestinal tract makes some sense. However, this is unlikely because TFM and niclosamide are dissolved in the water and would be directed across the gills during expiration. Like their freshwater teleost fish counterparts, drinking rates are low in larval lampreys (Barany et al., 2020), which also makes it unlikely that there would be appreciable ingestion of lampricide. Finally, there is no *a priori* reason to think that dissolved TFM or niclosamide would be extracted from the water. The feeding apparatus of the larval lamprey is designed to direct food particles (biofilm, algae, diatoms; Sutton and Bowen, 1994) trapped in mucus secreted by an endostyle, towards the esophageal opening (Rovainen, 1996; Mallatt, 1996), not dissolved materials, making this route highly unlikely as well.

Influence of alkalinity on lake sturgeon sensitivity to TFM – A case study

The relation between water alkalinity and gill microenvironment pH on TFM sensitivity may also help explain the greater probability of mortality seen in lake sturgeon during lampricide applications in waters of high alkalinity (Boogaard et al., 2003; O'Connor et al., 2017; Dobiesz et al., 2018). Chondrostean fishes such as the sturgeons and teleost fishes have evolved along separate lineages for the last 360 million or so years (Betancur-R et al., 2017). This has resulted in many differences in the physiology and body plan of the two groups, the most distinct being the presence of a cartilaginous skeleton and protrusible jaws in the chondrosteans as opposed to an ossified bony skeleton and fixed jaws in teleosts (Sallen, 2014). Less is known about differences in gill function between the two species, but the anatomy, ultrastructure and function of the gills appear to be more or less similar, playing key roles in not only gas exchange, but also acid-base and ion regulation (see Zydlewski and Wilkie, 2013 for review). The gross anatomy of the gills of sturgeons does differ in one respect from teleosts, however, which is the presence of a dorsolateral slit in the operculum of the gill cover (operculum), which may facilitate bi-directional respiratory flow of water across the gills when the animals are using suctorial feeding to obtain food from the bottom substrate of lakes and rivers (Burggren, 1978). This in turn may influence the dynamics of CO_2 excretion and acid-base equivalents across the gills, and how much acidification takes place in the gill microenvironment, which would affect TFM bioavailability.

Lake sturgeon are most vulnerable to TFM in their early life stages, when the 12-h LC_{50} of young-of-the-year (YOY) lake sturgeon intersects or even falls below the corresponding 12-h LC_{50} of larval sea lamprey (Boogaard et al., 2003; McDonald and Kolar, 2007). The greater sensitivity to TFM in these early life stages is related to higher rates of TFM uptake by smaller, younger animals,

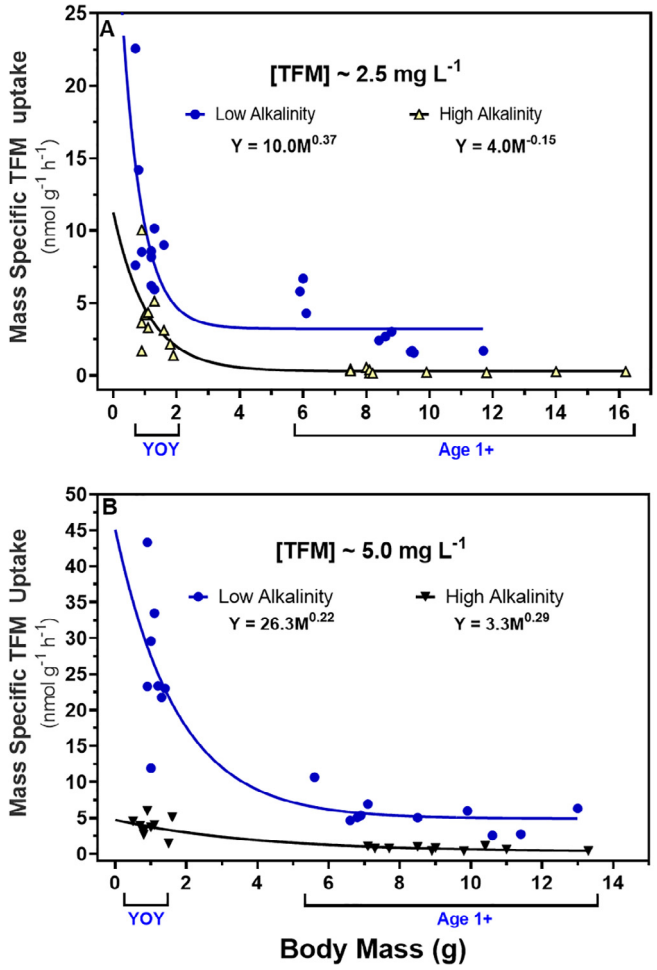

Fig. 7. TFM uptake decreases with body size and alkalinity. Rates of TFM uptake by smaller, young of the year (YOY) compared to larger, age 1 + lake sturgeon at low alkalinity (50 mg L^{-1} as $CaCO_3$) and high alkalinity (250 mg L^{-1} as $CaCO_3$), when exposed (A) 2.5 mg L^{-1} or (B) 5.0 mg L^{-1} TFM. Rates of TFM uptake were determined over 1 h using radioactively labeled ^{14}C-TFM (see Fig. 4 for further details). Curves of best fit for individual TFM uptake rates were calculated using least squares non-linear regression. Allometric power relations were calculated for each curve by plotting the log of whole animal TFM uptake (nmol h^{-1}) versus log body mass using Microsoft Excel. Data replotted from Hepditch et al. 2019. Refer to text for further detail.

in which mass specific rates of TFM uptake decrease exponentially as the animals grow and body mass increases (Fig. 7; Hepditch et al., 2019). This is likely due to the higher metabolic rates of smaller compared to larger sturgeons (Peake, 2005), which would be accompanied by higher rates of gill ventilation to deliver more oxygen to the gill, along with agents such as TFM for affected fish (Hepditch et al., 2019). An identical relation was observed between TFM uptake and body mass in larval sea lamprey (Tessier et al., 2018). The relation between body size and metabolic rate in animals can be described by the allometric power function of body mass, in which $Y = aM^b$, where Y represents an independent variable such as oxygen consumption (a proxy of metabolic rate), M is mass of the animal, the exponent b is a scaling or mass exponent which corrects for changes in Y with mass, and a is a normalization (or proportionality) constant (see Moyes and Schulte, 2008; Glazier, 2013 for reviews of this concept). This allometric relation also describes TFM uptake in lake sturgeon, but unlike the relation between oxygen consumption and body mass, where b is typically near 0.75 (Kleiber, 1947; Peters, 1983; Glazier, 2013), the corresponding value relating TFM uptake to body mass is smaller, more variable, and very strongly influenced by water alkalinity, pH, and TFM concentration. For this reason, it is not possible to derive a general power function for TFM uptake versus body size in lake sturgeon (or sea lamprey), as can be done when determining oxygen consumption versus body mass relations.

It is not yet clear if the ability of lake sturgeon to detoxify TFM differs with body size or age; however, ongoing studies comparing the sensitivity YOY lake sturgeon to more tolerant age one-year plus (1+) and older animals may soon answer this question. Previous studies have shown that the markedly lower capacity of sea lamprey to detoxify TFM by using the phase II biotransformation pathway of glucuronidation accounts for not only their greater sensitivity to TFM compared to non-target fishes, but variation in detoxification capacity also helps explain interspecific differences in TFM sensitivity (Lech and Statham, 1975; Kane et al., 1994).

Like sea lamprey and other non-target fishes, the sensitivity of juvenile (age 1+) lake sturgeon to TFM decreases with alkalinity (Fig. 8A), yet the probability of non-target mortality during lampricide applications is higher as water alkalinity increases (Fig. 8B; O'Connor et al., 2017). As described earlier, target treatment concentrations are directly proportional to alkalinity and pH (Bills et al., 2003; O'Connor et al., 2017). As Fig. 8B demonstrates, as TFM concentrations are increased, using either TFM or a TFM plus 1% niclosamide mixture, the probability of survival goes down. A key observation of O'Connor et al. (2017) was that the no observed effects concentration (NOEC) in lake sturgeon tended to be greater than the observed MLC for sea lamprey in lower alkalinity water, but at higher alkalinities, the NOEC was near or less than the sea lamprey MLC. These observations indicate that as alkalinity increases it increases the likelihood of survival more in sea lamprey than it does in lake sturgeon, at a given TFM concentration. To further examine this possibility, we used the raw data from O'Connor et al. (2017) to compare the MLC (12-h $LC_{99.9}$) of sea lamprey to that of the corresponding 12-h LC_{50} in the YOY lake sturgeon tested under identical water quality conditions. Not surprisingly, the resulting relation indicated that as the alkalinity was increased, both the 12-h LC_{50} and MLC values increased in a curvilinear fashion (decreasing sensitivity), as described earlier in sea lamprey (Fig. 9). Notably, with one exception, the 12-h LC_{50} values for lake sturgeon all fell near the 12-h $LC_{99.9}$ of sea lamprey plotted using the pH-alkalinity charts of Bills et al. (2003). However, when the sea lamprey MLC at pH 8.0 (near the measured pH values of the experiment) was multiplied by 1.4 times, to achieve TFM application target concentrations, the corresponding toxicity curve shifted upward, overlapping with that of the lake sturgeon. Thus, the combination of relatively low TFM tolerance in YOY lake sturgeon and the need to apply TFM at concentrations corresponding to 1.4 times the MLC, reduces the margin of safety between respective MLCs when lake sturgeon are exposed to TFM at concentrations that ensure sea lamprey mortality. *In situ*, O'Connor et al. (2017) found that survival was the lowest for YOY lake sturgeon (<100 mm) in the two rivers with pH > 8.12 and alkalinity >195 mg L^{-1} $CaCO_3$ (45 and 65%) compared with YOY survival rates of 80 to 100% for all other *in situ* lampricide applications. However, it should also be pointed out that attenuation and dilution downstream would lower the risk of TFM to sea lamprey and lake sturgeon as the distance from the application site increases. In other words, lake sturgeon located nearest the point of TFM application would likely be at greatest risk of non-target mortality.

One possible explanation for increased sensitivity of lake sturgeon to TFM at higher alkalinities is that the amount of acidification taking place at the gill surface is slightly greater than it is in sea lamprey, resulting in greater TFM bioavailability at the gill surface of lake sturgeon. For instance, if net CO_2 and metabolic acid production and excretion rates were higher in resident lake sturgeon compared to sea lamprey there would be more CO_2 and meta-

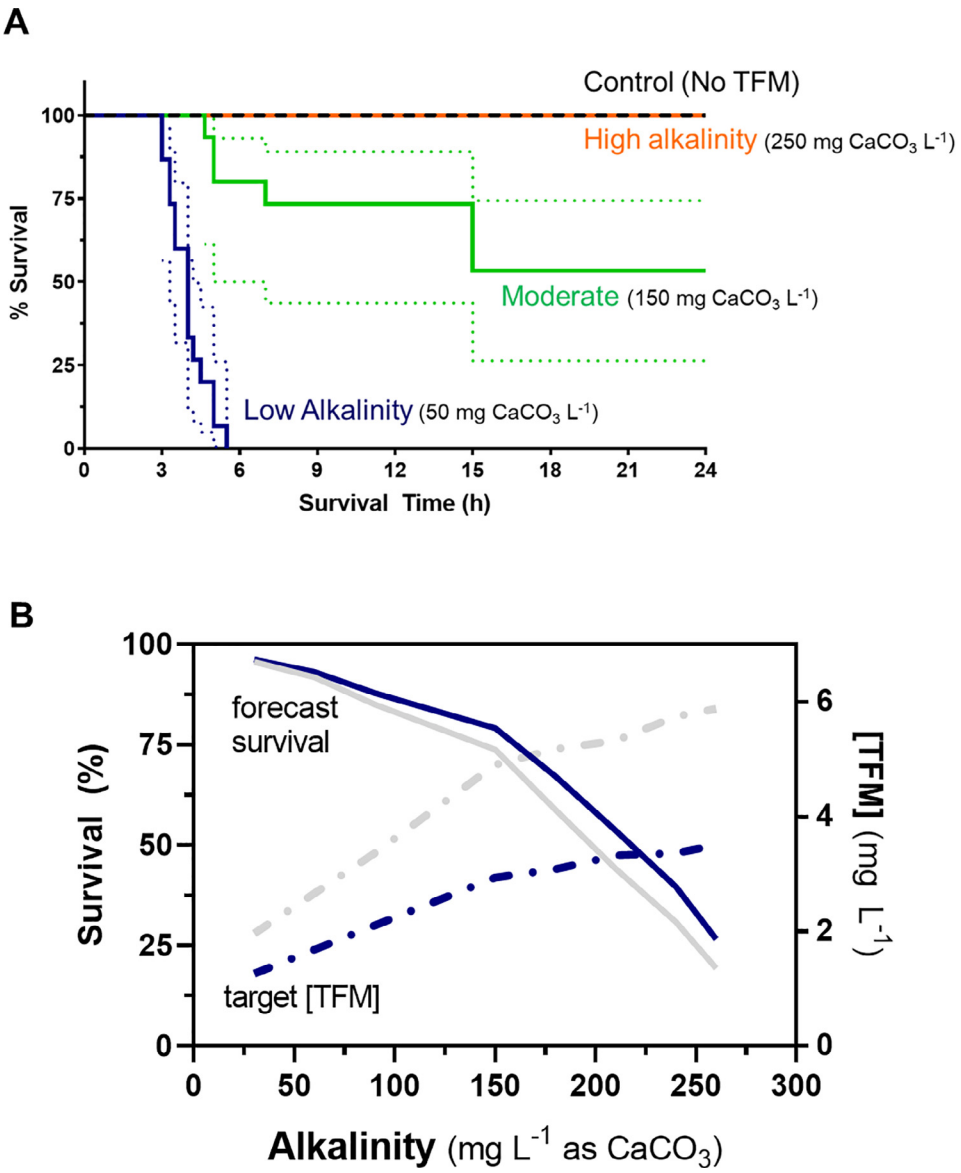

Fig. 8. Relation between lake sturgeon TFM sensitivity, alkalinity, and the MLC of sea lamprey. (A) Percent survival of age 1+ lake sturgeon to a nominal TFM concentration of 3.5 mg L^{-1} over 24 h (measured [TFM] = 3.61 ± 0.2) in waters of low (blue/dark line; 50 mg L^{-1}; N = 15), moderate (green/medium line; 150 mg L^{-1} $CaCO_3$), or high alkalinity (250 mg L^{-1} $CaCO_3$). Mortality was not observed in control (non-exposed) animals at any alkalinity (dashed line; N = 18 total). Dotted lines denote the calculated 95% confidence intervals. From Hepditch et al. (2019). (B) Forecast survival (solid lines) for lake sturgeon exposed to 1.4 times the sea lamprey LC99.9 (MLC) of TFM when exposed to TFM only (solid grey line) or a TFM plus 1% niclosamide mix (solid dark line). Target TFM concentrations for TFM only (dotted grey line) or TFM plus 1% niclosamide mixture (dotted dark line). Data were re-plotted from O'Connor et al. (2017; their Table 7). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

bolic H^+ excretion across the gills of the lake sturgeon, leading to a slightly lower pH at the surface of their gills. As a result, at a given concentration of TFM in the same bulk, higher alkalinity water, we would predict that there would be more un-ionized and less ionized TFM at the gill surface of the lake sturgeon compared to the sea lamprey in the same water, resulting in higher rates of TFM uptake and greater risk of mortality. A simple way to test this hypothesis would be to measure rates of TFM uptake in both sea lamprey and lake sturgeon at different alkalinities and TFM concentrations, in the same water, and relate it to the pH at the gill surface. As we saw earlier, even a slight acidification at the gill surface could be sufficient to increase the amount of bioavailable, unionized TFM, even in waters of moderate to high alkalinity.

To date, such experiments have not been performed. However, some insight is provided by the studies addressing the sensitivity of TFM uptake to changes in water TFM concentration in YOY and age 1 + lake sturgeon acclimated to low (50 mg L^{-1} as $CaCO_3$), moderate (150 mg L^{-1} as $CaCO_3$), and high alkalinity (250 mg L^{-1} as $CaCO_3$; Hepditch et al., 2019). This work demonstrated that the concentration dependent rates of TFM uptake decreased with alkalinity, but as the slopes of the TFM uptake versus concentration relation revealed, the rates of TFM uptake were less sensitive to changes in TFM concentration as alkalinity increased (i.e., flatter slope for the relation; Fig. 10). This may in fact explain why the toxicity of TFM (LC_{50} and $LC_{99.9}$) is almost insensitive to changes in TFM concentration as alkalinity surpasses 200 mg L^{-1} as $CaCO_3$ at a given water pH (Bills et al., 2003). Another notable observation was that the effect of water alkalinity on the overall sensitivity of TFM uptake to changes in external TFM concentration was much greater in the smaller, YOY fish compared the larger, age 1+ fish. Indeed, the slopes of the TFM uptake versus concentration relation were much steeper in the YOY versus age 1+ fish at each of the

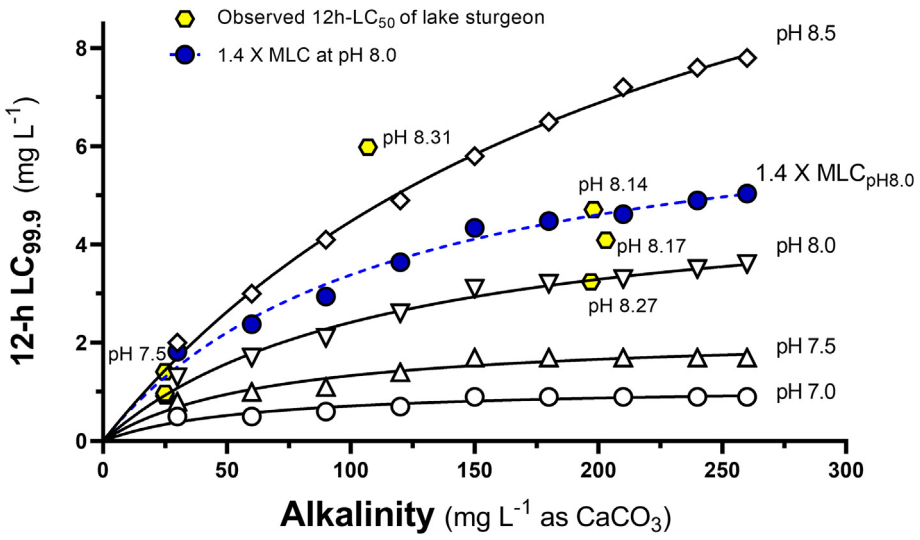

Fig. 9. The TFM sensitivity of lake sturgeon overlaps with the minimum lethal concentration (MLC) of sea lamprey. Figure comparing the relation between the TFM toxicity (12-h $LC_{99.9}$; MLC) of sea lamprey at different water alkalinities and pH (open symbols) to the observed 12-h LC_{50} of TFM to YOY lake sturgeon (solitary hexagons), including corresponding water pH in which the fish were tested. Data used in curves depicting the $LC_{99.9}$ versus alkalinity relation at each pH (Bills et al. 2003) were fitted by standard non-linear regression using the least squares fitting method (GraphPad Prism 8, San Diego, CA). Individual data points depicting the 12-h LC_{50} of lake sturgeon taken from O'Connor et al. (2017).

alkalinity. It was particularly notable that the slope of the relation in YOY fish was near unity at the highest alkalinity (m = 0.83) but approached zero (m = 0.178) in the age 1+ fish in higher alkalinity water. This greater sensitivity of TFM uptake to changes in TFM concentration in YOY lake sturgeon, even at higher alkalinity, might also explain why this life stage is so much more sensitive to TFM than their larger or older conspecifics (Fig. 10). Similar experiments comparing TFM uptake in larval sea lamprey to lake sturgeon exposed to increasing alkalinity and pHs, preferably the same water, are needed to test the hypothesis that the TFM uptake by YOY lake sturgeon at higher alkalinities is more sensitive to changes in external TFM concentration than in sea lamprey. Gill morphometry (e.g., surface area, blood-water diffusion distance) could also influence TFM uptake but would be expected to have less effect because it would not directly affect TFM bioavailability to the same degree as pH or alkalinity.

To understand why the TFM uptake versus alkalinity relation flattens at higher alkalinity, it is important to remember that TFM uptake takes place in its more bioavailable, un-ionized form. As noted above, the total TFM concentration (TFM_{total}) is the sum of un-ionized TFM (TFM-OH) plus ionized TFM ($TFM\text{-}O^-$). At higher alkalinities, where there is little acidification of the gill surface, the $TFM\text{-}O^-$ and TFM-OH concentration at the gill surface would be nearer the respective bulk water concentration. Because water pH typically increases with alkalinity, the proportion of TFM-OH of total TFM will be relatively low in the bulk water and at the gill surface. Hence, any increases in the total TFM concentration will be reflected by relatively small changes in TFM-OH in both the bulk water and at the gill surface, and therefore have little effect on TFM uptake. Whereas at lower alkalinities, where there will greater acidification at the gill surface due to the lower buffer capacity of the water, increases in the TFM_{total} in the bulk water will result in greater relative increases in TFM-OH bioavailability at the gill surface, and higher rates of TFM-uptake. In other words, as alkalinity increases, less acidification at the gill surface results in proportionally less TFM-OH formation relative to the total TFM concentration in the bulk water. However, as the pH increases further, towards values typical of higher alkalinity waters, there is relatively little change in the amount of TFM-OH with further increases in the total TFM concentration. This insensitivity to changes in TFM-OH concentration with total TFM, plus the absence or minimal acidification at the gill surface, would therefore keep TFM uptake levels low, even with relatively large steps in the total TFM concentration in the bulk water. In other words, relatively large increases in total water TFM concentration would be needed to achieve relatively small increments in the more bioavailable, un-ionized form of TFM at the sea lamprey gill surface. However, if the gill surface of lake sturgeon has even a slightly lower relative pH under these circumstances, the greater bioavailability of TFM could be fatal.

An additional or alternate possibility is that the buffer capacity of the blood influences the inwardly directed gradient for TFM-OH, leading to greater TFM uptake by lake sturgeon compared to sea lamprey. Lampreys lack Cl^-/HCO_3^- exchange proteins (anion exchange) on their red blood cells resulting in a much lower concentration of HCO_3^- in the plasma, plus they have a relatively low non-bicarbonate buffer capacity (Tufts and Boutilier, 1989; Nikinmaa et al., 1995). As result they have a lower capacity to control blood pH compared to most vertebrates, including sturgeons, in which plasma HCO_3^- concentrations and non-bicarbonate buffer capacity are at least 2–4 fold greater, resulting in more effective blood pH regulation (Tufts and Boutilier, 1989; Shartau et al., 2017). Because TFM accumulation results in greater reliance on anaerobic glycolysis, it would be expected to lead to decreases in tissue and blood pH due to the corresponding development of a metabolic acidosis, which is what is observed in the muscle of sea lamprey (Ionescu, 2020). If decreases in blood and muscle pH are in fact greater in sea lamprey than in YOY lake sturgeon due to their lower blood buffering capacity, then at a given external concentration of TFM, TFM-OH would be higher in blood of larval sea lamprey. This would result in a lower inwardly directed TFM-OH gradient in the sea lamprey than in the lake sturgeon, in which blood TFM-OH would be lower, resulting in a higher TFM-OH gradient, higher rates of TFM uptake and accumulation, and greater toxicity in the sturgeon. Further measurements of the pH and buffer capacity of the blood, along with further characterization of the gill microenvironment and measurements of TFM uptake would enable us to better understand this possibility.

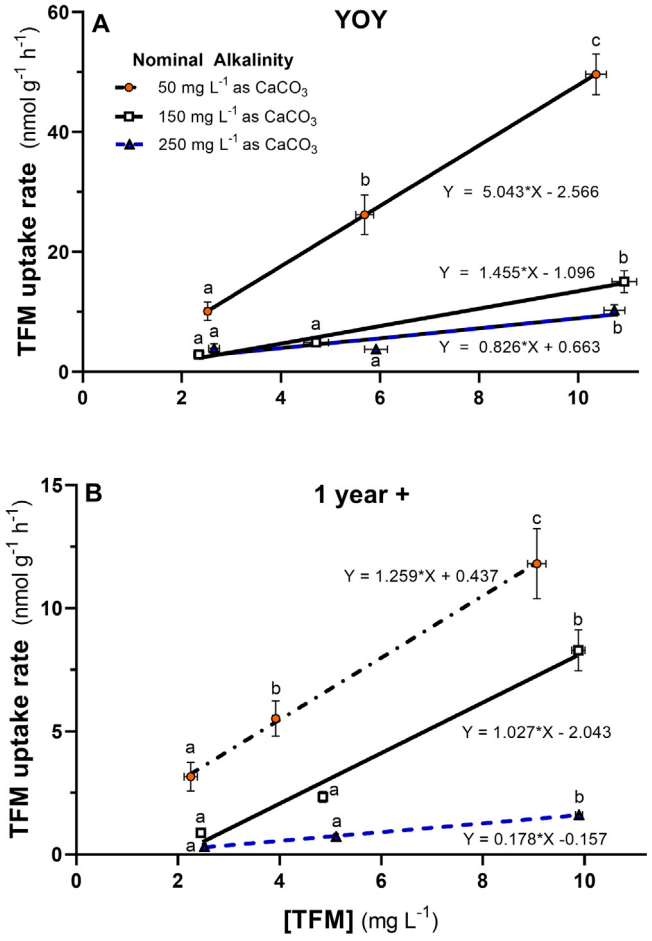

Fig. 10. TFM uptake is less sensitive to changes in TFM concentration at higher alkalinities. Changes in the rate of TFM uptake with TFM concentration in (A) young of the year (YOY) and (B) age 1+ lake sturgeon. Mean values for TFM uptake plotted against the measured TFM concentration, ±the standard error of the mean (SEM) for each (N = 8–10 per treatment). Lines of best fit determined by linear regression, with slopes within age groups differing significantly from one another. Mean TFM uptake rates with different lower-case letters are significantly different from one another at a given alkalinity and life stage ($P < 0.05$) as determined by ANOVA followed by Tukey's post-test. Data were replotted from Hepditch et al. (2019).

Perspectives

The effective control of sea lamprey populations in the Great Lakes will likely continue to rely on lampricides for the foreseeable future. The use of sea lamprey barriers, traps, and selective fish passage strategies may also continue to play a critical role in reducing lampricide requirements by preventing sea lamprey reproduction in the upstream reaches of many systems (Zielinski and Freiburger, 2021). Such facilities are expensive, take a long time to construct, and may face public or regulatory hurdles. Alternative control methods that are not yet ready to be implemented on a large scale, such as the use of pheromones for selectively guiding sea lamprey to traps remain in the experimental stages and are more likely to be used as a supplement to traditional SLC methods (Siefkes, 2017). The GLFC's social license to continue using lampricides also depends on the continued support of fisheries managers, government agencies, and the general public for what remains one of the most successful invasive species control programs in the world (Gaden et al., 2021). However, this support could be undermined by increasing concerns about the vulnerability of non-target organisms to lampricide toxicity.

Although lampricides have substantially contributed to the ongoing rehabilitation of the Great Lakes and surrounding watersheds (e.g., Lake Champlain, Vermont, New York, and Quebec; Finger Lakes, New York), a better understanding of how they work and of their potential adverse effects on non-target organisms is needed to use them more effectively and safely. While advances have been made to improve lampricide effectiveness, reduce lampricide use, and to protect non-target organisms, we are only beginning to understand the underlying physiological processes that determine how invasive sea lamprey and non-target organisms respond to TFM and niclosamide. The present synthesis reveals how complex interactions between pH and alkalinity influence TFM and niclosamide bioavailability at the gill, and how the effects of lampricides are further influenced by biotic factors such as life stage, body size, and respiration. As we demonstrate here, and in a companion paper on temperature and TFM (Hlina et al., 2021), the interactions between the biotic and abiotic factors that influence lampricide effectiveness and safety are much more complicated than previously realized.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are grateful to Joe Hunter, Sustainable Sturgeon Culture, and Dr. Gary Anderson, for providing us with the lake sturgeon that were crucial for our work. We also greatly appreciate the ongoing support provided by the Ontario Aqualture Research Centre (OARC), University of Guelph, Alma, Ontario, particularly Dr. Marcia Chiasson, Dr. Richard Mocchia, and Michael Burke, for holding and rearing the lake sturgeon used in our studies. We also thank the staff at the Hammond Bay Biological Station who provided the larval sea lamprey used in our studies. Special thanks to our students and past collaborators, whose hard work and contributions made this work possible. We also appreciate the efforts of three peer reviewers, who provided constructive suggestions and thorough edits of our original manuscript. Finally, we are grateful to Great Lakes Fishery Commission, who supported much of the previously published and the original research presented here through research contracts to MPW, in addition to hosting the Third Sea Lamprey International Symposium (SLIS III). Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

References

Adair, R., Sullivan, P., 2013. Sea lamprey control in the Great Lakes, 2012. Annual report to the Great Lakes Fishery Commission. Great Lakes Fishery Commission. Ann Arbor, MI.

Alasadi, A., Chen, M., Swapna, G.V.T., Tao, H., Guo, J., Collantes, J., Fadhil, N., Montelione, G.T., Jin, S., 2018. Effect of mitochondrial uncouplers niclosamide ethanolamine (NEN) and oxyclozanide on hepatic metastasis of colon cancer. Cell Death Dis. 9, 215.

Applegate, V.C., Howell, J.H., Moffett, J.W., Johnson, B.G.H., Smith, M.A., 1961. Use of 3-trifluoromethyl-4-nitrophenol as a selective sea lamprey larvicide. Great Lakes Fishery Commission Technical Report 1, 3–35.

Barany, A, Shaugnessy, C.A., Fuentes, J., Mancera, J.M., McCormick, S.D., 2020. Osmoregulatory role of the intestine in the sea lamprey (Petromyzon marinus). Am. J. Physiol. 318, R410–R417, 2020.

Barber, J., Steeves, M., 2019. Standard Operating Procedures for Application of Lampricides in the Great Lakes Fishery Commission Integrated Management of Sea Lamprey (Petromyzon marinus) Control Program. Great Lakes Fishery Commission SLC 04-001.10, Ann Arbor, MI.

Bartels, H., Potter, I.C., 2004. Review Cellular composition and ultrastructure of the gill epithelium of larval and adult lampreys Implications for osmoregulation in fresh and seawater. J. Exp. Biol. 207, 3447–3462.
Betancur-R, R., Wiley, E.O., Arratia, G., Acero, A., Bailly, N., Miya, M., Lecointre, G., Orti, G., 2017. 2017 Phylogenetic classification of bony fishes. BMC Evol. Biol. 17, 162. https://doi.org/10.1186/s12862-017-0958-3.
Bills, T.D., Marking, L.L., 1976. Toxicity of 3-trifluoromethyl-4-nitrophenol (TFM), 2'5-dichloro-4'-nitrosalicylanilide (Bayer 73) and a 98:2 mixture to fingerlings of seven fish species and to eggs and fry of coho salmon. U.S. Fish and Wildlife Service, Investigations in Fish Control No. 69.
Bills, T.D., Boogaard, M.A., Johnson, D.A., Brege, D.C., Scholefield, R.J., Westman, R.W., Stephens, B.E., 2003. Development of a pH/alkalinity treatment model for the applications of the lampricide TFM to streams tributary to the Great Lakes. J. Great Lakes Res. 29 (Suppl. 1), 510–520.
Birceanu, O., McClelland, G.B., Wang, Y.X.S., Wilkie, M.P., 2009. Failure of ATP supply to match ATP demand, the mechanism of toxicity of the lampricide, 3-trifluoromethyl-4-nitrophenol (TFM), used to control sea lamprey (*Petromyzon marinus*) populations in the Great Lakes. Aquat. Toxicol. 94, 265–274.
Birceanu, O., McClelland, G.B., Wang, Y.S., Brown, J.C.L., Wilkie, M.P., 2011. The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) uncouples mitochondrial oxidative phosphorylation in both sea lamprey (*Petromyzon marinus*) and TFM-tolerant rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. C 153, 342–349.
Birceanu, O., Sorensen, L.A., Henry, M., McClelland, G.B., Wang, Y.S., Wilkie, M.P., 2014. The effects of the lampricide 3-trifluoromethy1-4-nitrophenol (TFM) on fuel stores and ion balance in a non-target fish, the rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. C 160, 30–41.
Birceanu, O., Tessier, L.R., Huerta Buitrago, B., Li, W., McDonald, A., Wilkie, M.P., 2021. At the intersection between toxicology and physiology: What we have learned about sea lampreys and bony fish physiology from studying the mode of action of lampricides. J. Great Lakes Res. 47, S673–S689.
Boogaard, M.A., Bills, T.D., Johnson, D.A., 2003. Acute toxicity of TFM and a TFM/niclosamide mixture to selected species of fish, including lake sturgeon (*Acipenser fulvescens*) and mudpuppies (*Necturus maculosus*), in laboratory and field exposures. J. Great Lakes Res. 29 (Suppl. 1), 529–541.
Burggren, W.W., 1978. Gill ventilation in the sturgeon, *Acinpenser transmontanus*: unusual adaptations for bottom dwelling. Respir. Physiol. 34, 153–170.
Bussy, U., Chung-Davidson, Y.-W., Buchinger, T., Li, K., Smith, S.A., Jones, A.D., Li, W., 2018a. Metabolism of a sea lamprey pesticide by fish liver enzymes part A: identification and synthesis of TFM metabolites. Anal. Bioanal. Chem. 410, 1749–1761.
Bussy, U., Chung-Davidson, Y.-W., Buchinger, T., Li, K., Smith, S.A., Jones, A.D., Li, W., 2018b. Metabolism of a sea lamprey pesticide by fish liver enzymes part B: method development and application in quantification of TFM metabolites formed *in vivo*. Anal. Bioanal. Chem. 410, 1763–1774.
Christie, G.C., Adams, J.V., Steeves, T.B., Slade, J.W., Cuddy, D.W., Fodale, M.F., Young, R.J., Kuc, M., Jones, M.L., 2003. Selecting Great Lakes Streams for lampricide treatment based on larval sea lamprey surveys. J. Great Lakes Res. 29 (Suppl. 1), 152–160.
Clifford, A.M., Henry, M., Bergstedt, R., McDonald, D.G., Smits, A.S., Wilkie, M.P., 2012. Recovery of larval sea lampreys from short-term exposure to the pesticide 3trifluoromethyl-4-nitrophenol: implications for sea lamprey control in the Great Lakes. Trans. Am. Fish. Soc. 141, 1697–1710.
COSEWIC, 2017. COSEWIC Assessment and status report on the lake sturgeon (Acipenser fulvescens), Western Hudson Bay populations, Saskatchewan-Nelson River population, Southern Hudson Bay-James Bay population, Great Lakes-Upper St. Lawrence populations. Committee on the Status of Endangered Wildlife in Canada. Ottawa. 153 pp (Species at Risk Public registry website. Accessed February 17, 2021).
Dawson, V.K., 2003. Environmental fate and effects of the lampricide Bayluscide: a review. J. Great Lakes Res. 29 (Suppl. 1), 475–492.
Dawson, V.K., Gumming, K.B., Gilderhus, P.A., 1977. Efficacy of 3-trifluoromethyl-4-nitrophenol (TFM), 2,5-dichloro-4'-nitrosalicylanilide (Bayer 73) and a 98:2 mixtures as lampricides in laboratory studies. U.S. Department of the Interior, Fish and Wildlife Service. Invest. Fish Control 77, 1–11.
Dobiesz, N.E., Bence, J.R., Sutton, T., Ebener, M., Pratt, T.C., O'Connor, L.M., Steeves, T. B., 2018. Evaluation of sea lamprey-associated mortality sources on a generalized lake sturgeon population in the Great Lakes. J. Great Lakes Res. 44, 319–329.
Dymowska, A., Hwang, P.-P., Goss, G.G., 2012. Structure and function of ionocytes in the freshwater fish gill. Respir. Physiol. Neurobiol. 184 (2012), 282–292.
Erickson, R.J., McKim, J.M., Lien, G.J., Hoffman, A.D., Batterman, S.L., 2006. Uptake and elimination of ionizable organic chemicals at fish gills: I. Model formulation, parameterization, and behaviour. Environ. Toxicol. Chem. 25, 1512–1521.
Eshenroder, R.L., 2014. The Role of the Champlain Canal and Erie Canal as Putative Corridors for Colonization of Lake Champlain and Lake Ontario by Sea Lampreys. Trans. Am. Fish. Soc. 143, 634–649.
Evans, D.H., Piermarini, P.M., Choe, K.P., 2005. The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. Physiol. Rev. 85, 97–177.
Ferreira-Martins, D., Wilson, J.M., Kelly, S.P., Kolosov, D., McCorkick, S.D., 2021. A review of osmoregulation in lamprey. J. Great Lakes Res. 47, S59–S71.
Fodale, M.F., Bergstedt, R.A., Cuddy, D.W., Adams, J.V., Stolyarenko, D.A., 2003. Planning and executing a lampricide treatment of the St. Mary's River using georeferenced data. J. Great Lakes Res. 29 (Suppl. 1), 706–716.
Frazier, B.E., Naima, T.J., Sandheinrich, M.B., 1996. Temporal and vertical of total ammonia nitrogen and un-ionized ammonia nitrogen in sediment pore water from the Upper Mississipi River. Environ. Toxicol. Chem. 15, 92–99.
Gaden, M., Brant, C.O., Stedman, R.C., Cooke, S.J., Young, N., Lauber, T.B., Nguyen, V. M., Connelly, N.A., Knuth, B., 2021. Shifting baselines and social license to operate: Challenges in communicating sea lamprey control. J. Great Lakes Res. 47, S800–S808.
Glazier, J.F., 2013. Log-transformation is useful for examining proportional relationships in allometric scaling. J. Theor. Biol. 334, 200–203.
Golder Associates Ltd., 2011. Recovery Strategy for Lake Sturgeon (*Acipenser fulvescens*) – Northwestern Ontario, Great Lakes-Upper St. Lawrence River and Southern Hudson Bay-James Bay populations in Ontario. Ontario Recovery Strategy Series. Prepared for the Ontario Ministry of Natural Resources. vii + 77 pp.
GLFC, 2011. Strategic Vision of the Great Lakes Fishery Commission (GLFC) 2011-2020. 29 pages.
Harkness, W.J.K., Dymond, J.R., 1961. The lake sturgeon: the history of its fishery and problems of conservation. Ontario Department of Lands and Forestry, Fish and Wildlife Branch, Toronto, ON.
Henry, M., Birceanu, O., Clifford, A.M., McClelland, G.B., Wang, Y.S., Wilkie, M.P., 2015. Life stage dependent responses to the lampricide, 3-trifluoromethy1-4-nitrophenol (TFM), provide insight into glucose homeostasis and metabolism in the sea lamprey (*Petromyzon marinus*). Comp. Biochem. Physiol. C 169, 35–45.
Hepditch, S.L., Tessier, L.R., Wilson, J.M., Birceanu, O., O'Connor, L.M., Wilkie, M.P., 2019. Mitigation of lampricide toxicity to juvenile lake sturgeon: The importance of water alkalinity and life stage. Conserv. Physiol. 7. 0.1093/conphys/coz089.
Hepditch, S., Birceanu, O., Wilkie, M.P., 2021. Chemical control of invasive sea lamprey: Using the toxic unit and additive index approaches to understand the interactions of two piscicides, TFM and niclosamide. Environ. Toxicol. Chem. 40, 1419–1430.
Hill, R.W., Wyse, G.A., 1989. Animal Physiology –. Harper and Row Publishing, New York.
Hlina, B.L., Tessier, L.R., Wilkie, M.P., 2017. Effects of water pH on the uptake and elimination of the piscicide, 3-trifluoromethyl-4-nitrophenol (TFM), by larval sea lamprey. Comp. Biochem. Physiol. C 200, 9–16.
Hlina, B.L., Birceanu, O., Robinson, C.S., Dhiyebi, H., Wilkie, M.P., 2021. The relationship between thermal physiology and lampricide sensitivity in larval sea lamprey (Petromyzon marinus). J Great Lakes R. 47, S272–S284.
Hubert, T.D., 2003. Environmental fate and effects of the lampricide TFM: a review. J. Great Lakes Res. 29, 456–474.
Huerta, B., Chung-Davidson, Y.-W., Bussy, U., Zhang, Y., Bazil, J.N., Li, W., 2020. Sea lamprey cardiac mitochondrial bioenergetics after exposure to TFM and its metabolites. Aquat. Toxicol. 219, 105380.
Hughes, G.M., 1984. General anatomy of the gills. In: Hoar, W.S., Randall, D.J. (Eds.), Fish Physiology. Academic Press, pp. 1–72.
Hunn, J.B., Allen, J.L., 1974. Movement of drugs across gills of fishes. Annu. Rev. Pharmacol. Toxicol. 14, 47–55.
Ionescu, R.A., 2020. Physiological Impacts of Lampricides on Invasive Sea Lamprey (*Petromyzon marinus*) and Non-target Fishes Ph.D. thesis. Wilfrid Laurier University, Department of Biology.
Ionescu, R.A., Hepditch, S., Wilkie, M.P., 2021. The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) causes temporary metabolic disturbances in juvenile lake sturgeon (Acipenser fulvescens): Implications for sea lamprey control and fisheries conservation. Conservation Physiol. MS ID CONPHYS, 2021–2024. in press.
Jurgeit, A., McDowell, R., Moese, S., Meldrum, E., Schwendener, R., Greber, U.F., 2012. Niclosamide is a proton carrier and targets acidic endosomes with broad antiviral effects. PLoS Pathog. 8.
Kanayama, R.K., 1963. The use of alkalinity and conductivity measurements to estimate concentrations of 3-trifluoromethyl-4-nitrophenol required for treating lamprey streams. Great Lakes Fishery Commission Technical Report No. 7. Great Lakes Fisheries Commission, Ann Arbor, MI.
Kane, A.S., Kahng, M.W., Reimschuessel, R., 1994. UDP-glucoronyltransferase kinetics for 3-trifluoromethyl-4-nitrophenol (TFM) in fish. Trans. Am. Fish. Soc. 123, 217–222.
Kleiber, M., 1947. Body size and metabolic rate. Physiol. Rev. 27, 511–541.
Köhler, P., 2001. The biochemical basis of anthelmintic action and resistance. Int. J. Parasitol. 31, 336–345.
Lardans, V., Dissous, C., 1998. Snail control strategies for reduction of schistosomiasis transmission. Parasitol. Today 14, 413–417.
Lawrie, A.H., 1970. The sea lamprey in the Great Lakes. Trans. Am. Fish. Soc. 99, 766–775.
Lech, J.J., Statham, C., 1975. Role of glucoronide formation in the selective toxicity of 3-trifluoromethyl-4-nitrophenol (TFM) for the sea lamprey: comparative aspects of TFM uptake and conjugation in sea lamprey and rainbow trout. Toxicol. Appl. Pharmacol. 31, 150–158.
Lewis, S.V., 1980. Respiration of lampreys. Can. J. Fish. Aquat. Sci. 37, 1711–1722.
Mallatt, J., 1996. Ventilation and the origin of jawed vertebrates: A new mouth. Zool. J. Linn. Soc. 117, 329–404.
Manzon, R., Youson, J., Holmes, J., 2015. Lamprey metamorphosis. In: Docker, M. (Ed.), Lampreys: Biology, Conservation and Control. Springer Dordrecht. Fish Fisheries Science 37. pp. 139–214.
McConville, M.B., Hubert, T.D., Remucal, C.K., 2016. Direct photolysis rates and transformation pathways of the lampricides TFM and niclosamide in simulated sunlight. Environ. Sci. Technol. 50, 9998–10006.

McConville, M.B., Cohen, N.M., Nowicki, S.M., Lantz, S.R., Hixson, J.L., Ward, A.S., Remucal, C.K., 2017a. A field analysis of lampricide photodegradation in Great Lakes tributaries. Environ. Sci. Process. Impacts 19, 891–900.
McConville, M.B., Mexyk, S.P., Lantz, S.R., Remucal, C.K., 2017b. Indirect photodegradation of the lampricides TFM and niclosamide. Environ. Sci. Process. Impacts 19, 1028–1039.
McDonald, D.G., Kolar, C.S., 2007. Research to guide the use of lampricides for controlling sea lamprey. J. Great Lakes Res. 33, 20–34.
McLaughlin, R.L., Hallett, A., Pratt, T.C., O'Connor, L.M., McDonald, D.G., 2007. Research to guide use of barriers, traps, and fishways to control sea lamprey. J. Great Lakes Res. 33, 7–19.
Moyes, C.D., Schulte, P.M., 2008. Principles of Animal Physiology. Pearson-Benjamin Cummings, Toronto, ON.
Newman, M.C., Unger, M.A., 2003. Fundamentals of Ecotoxicology. Lewis Publishers, New York.
Niblett, P.D., Ballantyne, J.S., 1976. Uncoupling of oxidative phosphorylation in rat liver mitochondria by the lamprey larvicide TFM (3-trifluoromethyl-4-nitrophenol). Pestic. Biochem. Physiol. 2, 103–120.
Nikinmaa, M., Airaksinen, S., Virkki, L.V., 1995. Haemoglobin function in intact lamprey erythrocytes: interactions with membrane function in the regulation of gas transport and acid-base balance. J. Exp. Biol. 198, 2423–2430.
O'Connor, L.M., Pratt, T.C., Steeves, T.B., Stephens, B., Boogaard, M., Kaye, C., 2017. In situ assessment of lampricide toxicity to age-0 lake sturgeon. J. Great Lakes Res. 43, 189–198.
Park, S.J., Shin, J.H., Kang, H., Hwang, J.J., Cho, D.H., 2011. Niclosamide induces mitochondria fragmentation and promotes both apoptotic and autophagic cell death. BMB Reports 44, 517–522.
Peake, S.J., 2005. Swimming and Respiration. In: Sturgeons and paddlefish of North America. In: LeBreton, G.T.O., Beamish, F.W.H., McKinley, R.S. (Eds.), Fish and Fisheries Series, vol. 27. Springer, Dordrecht, pp. 147–166.
Peters, R.H., 1983. Ecological Implications of Body Size. Cambridge Studies in Ecology. Cambridge University Press, Cambridge, UK.
Playle, R.C., Wood, C.M., 1989. Water chemistry changes in the gill microenvironment of rainbow trout – experimental observations and theory. J. Comp. Physiol. B. 5, 527–537.
Pollock, M.S., Carr, M., Kreitals, N.M., Phillips, I.D., 2015. Review of a species in peril: what we do not know about lake sturgeon may kill them. Environ. Rev. 23, 30–43.
Potter, I., Welsch, U., Wright, G.M., Honma, Y., Chiba, A., 1995. Light and electron microscope studies of the dermal capillaries in three species of hagfishes and three species of lampreys. J. Zool. (London) 235, 677–688.
Pratt, T.C., Morrison, B.J., Quinlan, H.R., Elliott, R.F., Grunder, S.A., Chiotti, J.A., Young, B.A., 2021. Implications of the sea lamprey control program for lake sturgeon conservation and rehabilitation efforts. J. Great Lakes Res. 47, S421–S429.
Randall, D.J., 1972. Respiration. Pages 287–306 in The Biology of Lampreys. Hardistry, M.W., Potter I.C. Academic Press, London.
Reis-Santos, P., McCormick, S.D., Wilson, J.M., 2008. Ionoregulatory changes during metamorphosis and salinity exposure of juvenile sea lamprey (*Petromyzon marinus* L.). J. Exp. Biol. 211, 978–988.
Rovainen, C.M., 1996. Feeding and breathing in lampreys. Brain Behav. Evol. 48, 297–305.
Sallen, L.C., 2014. Major issues in the origins of ray-finned fish (Actinopterygii) biodiversity. Biol. Rev. 89, 950–971. https://doi.org/10.1111/brv.12086.
Scholefield, R.J., Bergstedt, R.A., Bills, T.D., 2003. Relation of concentration and exposure time to the efficacy of niclosamide against larval sea lampreys (*Petromyzon marinus*). J. Great Lakes Res. 29 (Suppl. 1), 493–499.
Seelye, J.G., Johnson, D.A, Weise, J.G., King, E.L. Jr., 1988. Guide for determining application rates of lampricides for control of sea lamprey ammocoetes. Great Lakes Fishery Commission Technical Report No. 52. Great Lakes Fishery Commission, Ann Arbor, MI.
Shartau, R.B., Baker, D.W., Brauner, C.J., 2017. White sturgeon (*Acipenser transmontanus*) acid-base regulation differs in response to different types of acidosis. J. Comp. Physiol. B. 187, 985–994.
Siefkes, M.J., 2017. Use of physiological knowledge to control the invasive sea lamprey (*Petromyzon marinus*) in the Laurentian Great Lakes. Conserv. Physiol. 5.
Smith, B.R., Tibbles, J.J., 1980. Sea lamprey (*Petromyzon marinus*) in Lakes Huron, Michigan and Superior – history of invasion and control, 1936–78. Can. J. Fish. Aquat. Sci. 37, 1780–1801.
Sullivan W.P., Burkett D.P., Boogaard M.A., Criger L.A., Freiburger C.E., Hubert T.D., Leistner K.G., Morrison B.J., Nowicki S.M., Robertson S.N.P., Rowlinson A.K., Scotland B.J., Sullivan, T.B., 2021. Advances in the use of lampricides to control sea lampreys in the Laurentian Great Lakes, 2000-2019. J. Great Lakes Res. 47, S216–S237.
Sunga, J., Wilson, J.M., Wilkie, M.P., 2020. Functional re-organization of the gills of metamorphosing sea lamprey (Petromyzon marinus): Preparation for a blood diet and the fresh water to sea water transition. Journal of Comparative Physiology B. In Press.
Sutton, T.M., Bowen, S.H., 1994. Significance of organic detritus in the diet of larva lampreys in the Great Lakes basin. Can. J. Fish. Aquat. Sci. 51, 2380–2387.
Tessier, L.R., Long, T.A.F., Wilkie, M.P., 2018. Influence of body size, metabolic rate and life history stage on the uptake and excretion of the lampricide 3-trifluoromethyl-4nitrophenol (TFM) by invasive sea lampreys (*Petromyzon marinus*). Aquat. Toxicol. 194, 27–36.
Tufts, B., Boutilier, R., 1989. The absence of rapid chloride/bicarbonate exchange in lamprey erythrocytes: implications for CO2 transport and ion distributions between plasma and erythrocytes in the blood of *Petromyzon marinus*. J. Exp. Biol. 144, 565–576.
Van Den Bossche, H., 1985. How anthelmintics help us to understand helminths. Parasitology 90, 675–686.
Viant, M.R., Walton, J.H., Tjeerdema, R.S., 2001. Comparative sublethal actions of 3-trifluoromethyl-4-nitrophenol in marine molluscs, as measured by in vivo P-31 NMR. Pestic. Biochem. Physiol. 71, 40–47.
Welsh, A., Mohr, L., Boase, J., 2017. Identifying conservation hotspots in non-breeding areas: a case study of lake sturgeon (*Acipenser fulvescens*) in the Great Lakes. Biodivers. Conserv. 26, 931–941.
Wetzel, R.G., 1983. Limnology. CBS College Publishing, New York.
Wilkie, M.P., 2002. Ammonia excretion and urea handling by fish gills: Present understanding and future research challenges. J. Exp. Zool. 293, 284–301.
Wilkie, M.P., 2011. Lampreys: environmental physiology. In: Farrell, A.P. (Ed.), Encyclopedia of Fish Physiology: From Genome to Environment, vol. 3. Academic Press. San Diego, CA, pp. 1788–1799.
Wilkie, M.P., Bradshaw, P.G., Joanis, V., Claude, J.F., Swindell, S.L., 2001. Rapid metabolic recovery following vigorous exercise in burrow-dwelling larval sea lampreys (*Petromyzon marinus*). Physiol. Biochem. Zool. 74, 261–272.
Wilkie, M.P., Holmes, J.A., Youson, J.H., 2007. The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) interferes with intermediary metabolism and glucose homeostasis, but not with ion balance, in larval sea lamprey (*Petromyzon marinus*). Can. J. Fish. Aquat. Sci. 64, 1174–1182.
Wilkie, M.P., Hubert, T.D., Boogaard, M.A., Birceanu, O., 2019. Control of invasive sea lampreys using the piscicides TFM and niclosamide: Toxicology, successes & future prospects. Aquat. Toxicol. 211, 235–252.
Wilson, J.M., Laurent, P., 2002. Fish gill morphology: inside out. J. Exp. Zool. 293, 192–213.
Wright, P.A., Heming, T., Randall, D.J., 1986. Downstream pH changes in water flowing over the gills of rainbow trout. J. Exp. Biol. 126, 499–512.
Youson, J.H., 2003. The biology of metamorphosis in sea lampreys: Endocrine, environmental, and physiological cues and events, and their potential application to lamprey control. J. Great Lakes Res. 29 (suppl. 1), 26–49.
Zhao, Q.P., Xiong, T., Xu, X.J., Jiang, M.S., Dong, H.F., 2015. De Novo Transcriptome Analysis of *Oncomelania hupensis* after Molluscicide Treatment by Next-Generation Sequencing: Implications for Biology and Future Snail Interventions. PLoS ONE 10.
Zielinski, D.P., Freiburger, C., 2021. Advances in fish passage in the Great Lakes basin. J. Great Lakes Res. 47, S439–S447.
Zydlewski, J., Wilkie, M.P., 2013. Freshwater to seawater transitions in migratory fishes. Pages 253-326 in Fish Physiology, Volume 32. Euryhaline Fishes. SD McCormick, AP Farrell and CJ Brauner (eds). Elsevier, London.