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PERSPECTIVE

# Rapid evolution meets invasive species control: the potential for pesticide resistance in sea lamprey

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**Abstract:** Rapid evolution of pest, pathogen, and wildlife populations can have undesirable effects, for example, when insects evolve resistance to pesticides or fishes evolve smaller body size in response to harvest. A destructive invasive species in the Laurentian Great Lakes, the sea lamprey (*Petromyzon marinus*) has been controlled with the pesticide 3-trifluoromethyl-4-nitrophenol (TFM) since the 1950s. We evaluated the likelihood of sea lamprey evolving resistance to TFM by (*i*) reviewing sea lamprey life history and control; (*ii*) identifying physiological and behavioural resistance strategies; (*iii*) estimating the strength of selection from TFM; (*iv*) assessing the timeline for evolution; and (*v*) analyzing historical toxicity data for evidence of resistance. The number of sea lamprey generations exposed to TFM was within the range observed for fish populations where rapid evolution has occurred. Mortality from TFM was estimated as 82%–90%, suggesting significant selective pressure. However, 57 years of toxicity data revealed no increase in lethal concentrations of TFM. Vigilance and the development of alternative controls are required to prevent this aquatic invasive species from evolving strategies to evade control.

**Résumé :** L'évolution rapide de populations d'organismes nuisibles, de pathogènes et d'animaux sauvages peut avoir des effets indésirables comme, par exemple, des insectes qui développent une résistance aux pesticides ou une diminution de la taille du corps des poissons en réponse à la pêche. Le pesticide 3-trifluorométhyl-4-nitrophénol (TFM) est utilisé depuis les années 1950 pour lutter contre la lamproie (*Petromyzon marinus*), une espèce envahissante destructrice dans les Grands Lacs laurentiens. Nous avons évalué la probabilité que les lamproies développent une résistance au TFM par (*i*) l'examen du cycle biologique des lamproies et des méthodes de lutte contre cette espèce, (*ii*) la détermination des stratégies de résistance physiologiques et comportementales (*iii*) l'estimation de la force de la sélection découlant du TFM, (*iv*) l'évaluation de la durée nécessaire pour qu'il y ait évolution et (*v*) l'analyse de données historiques sur la toxicité pour voir s'il existe des indices de résistance. Le nombre de générations de lamproies exposées au TFM est dans la fourchette observée pour les populations de poissons ayant subi une évolution rapide. La mortalité causée par le TFM est estimée à 82–90 %, ce qui indiquerait une pression de sélection significative. Cependant, 57 années de données sur la toxicité ne révèlent aucune augmentation des concentrations létales de TFM. La vigilance et la mise au point d'autres mesures de lutte sont nécessaires pour empêcher cette espèce aquatique envahissante de développer des stratégies lui permettant de contrer les efforts de lutte. [Traduit par la Rédaction]

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## Introduction

The capability of pathogens and pests to evolve resistance to control measures is a global concern with implications for human health, natural resource management, and agriculture (Palumbi 2001). Resistance to various chemicals has been documented in many taxa (Davies and Davies 2010; Mota-Sanchez et al. 2002; Wirgin and Waldman 2004; www.pesticideresistance.org). Further, the evolution of resistance can result in substantial economic loss to individuals, industry, and governments (Howard et al. 2003; Howard and Scott 2005; Alsan et al. 2015). Insecticide-resistant mosquitos carrying the malaria vector in Africa provide a prominent example involving major human suffering (Enayati and Hemingway 2010). Recognition of the widespread occurrence of resistance and its consequences is prompting the development of specific strategies targeted at slowing or preventing its occurrence (Tabashnik et al. 2014).

In fisheries, aquatic invasive species are non-native pests that threaten aquatic ecosystems. One of the most notorious aquatic invasive species is the sea lamprey (*Petromyzon marinus*), which precipitated a catastrophic collapse of economically valuable and culturally important fisheries resources when it invaded the Laurentian Great Lakes (Siefkes et al. 2013). Sea lamprey were first recorded in Lake Ontario in 1888 (Eshenroder 2014) and by 1938 had spread to each of the Great Lakes (Christie and Goddard 2003). This invasion was followed by massive declines and the extirpation of several native fish populations (Siefkes et al. 2013).

The chemical pesticide 3-trifluoromethyl-4-nitrophenol (TFM) has been applied to Great Lakes tributaries to control sea lamprey since the 1950s (Siefkes et al. 2013). TFM is applied as a lampricide to selectively target and kill larval sea lamprey while leaving many other aquatic organisms unharmed (Applegate et al. 1957, 1958). The application of TFM expanded between the 1950s and 1980s (Table 1), contributing to substantial reductions in sea lamprey abundance (Lawrie 1970; Heinrich et al. 2003). A second pesticide, 2′,5-dichloro-4′-nitrosalicylanilide (aka. niclosamide, Bayluscide), originally used as a molluscicide, was discovered in 1964 as having synergistic properties with TFM (Howell et al. 1964) and was subsequently applied in small doses to treat select tributaries. Application of these two pesticides (also referred to as lampricides), and in particular TFM, remains the cornerstone of sea lamprey control today and has supported recoveries of formerly depleted fish stocks, such as Lake Superior lake trout (*Salvelinus namaycush*), highly valued for their contribution to the health and economy of the Great Lakes and their cultural importance to First Nations communities (Smith and Tibbles 1980; Christie and Goddard 2003; Goddard 2013).

In this paper, we evaluate the possibility that Great Lakes sea lamprey could evolve resistance to the pesticides used to control them. As would be expected from any source of substantial mortality acting on a population, the application of lampricides over the past four to six decades has potentially imposed considerable selective pressure on sea lamprey. Pesticide resistance has evolved in diverse taxa, including bacteria, insects, and vertebrates, following continued exposure to a toxin, pesticide, or contaminant. While the generation time of sea lamprey (>4 years; see discussion below) is considerably longer than the generation times of bacteria (hours–days) or arthropod pests (days–months), numerous examples of rapid evolution contributing to human-induced trait changes in fish and wildlife populations highlight the potential threat and warrant consideration (Palkovacs et al. 2012; Sharpe and Hendry 2009).

Resistance to lampricides would lead to reduced effectiveness of sea lamprey control, resulting in increased mortality of commercial, sport, and culturally important fish populations in the Great Lakes. Furthermore, once resistance evolves it may be slow to reverse depending on associated costs or trade-offs. In fish, evolutionary recovery following the removal of anthropogenic selection has a slow reverse trajectory (Conover et al. 2009; Enberg et al. 2009), coined a "Darwinian debt", to be paid by future generations (U. Dieckmann, Financial Times, 28 August 2004). However, although resistance leads to increased fitness in the presence of the chemical, it could cause reduced fitness under normal conditions; in such a case, resistance might be expected to reverse relatively quickly once the chemical is removed (Levinton et al. 2003; Mackie et al. 2010). Evaluating the likelihood of sea lamprey resistance to lampricides has broader implications for management and control of invasive species, particularly vertebrate aquatic invasive species, by providing insights on rapid evolution in animal populations and on how applied evolutionary biology can inform management of pressing global issues (Hendry et al. 2011; Carroll et al. 2014).

**Table 1.** The number of generations of sea lamprey exposed to the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) in the Great Lakes given the timeline of lampricide application.

| Lake | Year of first application of TFM | No. of generations of sea lamprey exposed to TFM | Source |
|---|---|---|---|
| Superior | 1958 | 11.6 | Heinrich et al. 2003 |
| Huron | 1960 | 11.2 | Morse et al. 2003 |
| Michigan | 1960 | 11.2 | Lavis et al. 2003*b* |
| Ontario | 1971 | 9.0 | Larson et al. 2003 |
| Erie | 1986 | 6.0 | Sullivan et al. 2003 |

**Note:** Sea lamprey generation time was assumed to be 5 years.

Our synthesis of lampricide resistance includes (1) a review of sea lamprey life history and control in the Great Lakes; (2) identifying the opportunities for resistance to develop in sea lamprey, including physiological and behavioural strategies; (3) estimates of the strength of selection from lampricides; (4) assessment of whether sufficient time has passed for any evolution to occur; (5) analysis of lampricide toxicity data to look for signs of resistance; (6) identifying strategies for reducing the chances of resistance; and (7) conclusions on the implications our synthesis has for the control and management of other invasive species.

## Sea lamprey life history and control in the Great Lakes

Following metamorphosis and prior to maturation, sea lamprey are parasites — attaching to their fish hosts using an oral disc, puncturing the skin and muscle of the fish with their rasping tongue, and ingesting the blood and body fluids of the living animal (Renaud et al. 2009). Sea lamprey spend 12–18 months in this parasitic phase in the open waters of the Great Lakes before maturing and migrating into tributaries to spawn (Bergstedt and Swink 1995). Sea lamprey do not show natal homing behaviour (Bergstedt and Seelye 1995; Waldman et al. 2008), instead relying on odorants emitted by lamprey larvae to find suitable tributaries for spawning (Fine et al. 2004; Buchinger et al. 2013). Sea lamprey fecundity varies geographically within the Great Lakes, averaging greater than 50 000 eggs per female (Smith and Marsden 2007; Dawson et al. 2015). Fecundity is considered to be relatively high compared with other fishes, but fertilized eggs are vulnerable to predation and annual recruitment variability is high (Jones et al. 2003; Dawson et al. 2015). Following spawning, adults rapidly senesce and die (Johnson et al. 2015).

After hatching, larvae drift downstream and burrow into stream sediments where they remain as filter feeders for the majority of their life until reaching a critical length and condition factor in the fall preceding metamorphosis (Holmes and Youson 1994; Manzon et al. 2015). This ensures that they have sufficient lipid reserves to undergo the nonfeeding period of metamorphosis the following summer (Manzon et al. 2015). In the Great Lakes, age at metamorphosis is reported to vary from 3 to 7 years (Potter 1980), but interpretation of these values is complicated by the chal-

lenges of accurately estimating sea lamprey age and by the variation in age at metamorphosis among tributaries and among individuals within a cohort (Dawson and Jones 2009; Potter 1980; Potts et al. 2015).

Sea lamprey do not return to natal tributaries to spawn, which causes genetic mixing during reproduction of individuals born in different tributaries; this feature of sea lamprey life history constrains the extent of local adaptation. However, lack of genetic differentiation at a local scale does not preclude the possibility of weak spatial stock structure or subtle genetic differences among sea lamprey residing far apart in geographically different regions of the Great Lakes (e.g., Hess et al. 2013; Lanca et al. 2014; Krabbenhoft and Dowling 2015). For example, a higher probability of spawning between individuals from the tributaries of a given lake could create some weak spatial stock structure even though some mixing also occurs.

The Great Lakes Fishery Commission (GLFC) oversees sea lamprey control in the Great Lakes (reviewed in Siefkes et al. 2013). In the 1950s, initial attempts to control sea lamprey involved using traps and weirs to target adults migrating into spawning tributaries (McLain et al. 1965). The discovery that TFM could selectively kill larval sea lamprey (Applegate et al. 1957, 1958) led to its first application in Lake Superior in 1958, with full expansion of the program to the other lakes by the 1980s (Table 1). After its discovery in 1964, niclosamide was incorporated in lesser amounts (1%–2%) into some TFM applications (Howell et al. 1964). The toxicity of niclosamide is not as species-specific, but can reduce the amount of TFM required for successful treatment. A granular formulation of niclosamide is also used in some deep tributaries and lentic areas where the application of TFM is not practical (Siefkes et al. 2013). Barriers placed into tributaries have also contributed to sea lamprey control by blocking upstream spawning migration and restricting access to spawning and larval habitat (McLaughlin et al. 2013). Other control measures have been carried out to a lesser degree, including trapping and release of sterile male sea lamprey (Christie and Goddard 2003). Lampricide application, in particular the continued use of TFM, is the foundation of sea lamprey control in the Great Lakes, both historically and for the foreseeable future.

Only 500 of the approximately 5340 Great Lakes tributaries have produced sea lamprey, and 336 of these have been treated at least once with lampricide (Adair and Sullivan 2014*a*). Tributaries do not require treatment on an annual basis because of the time required by larval sea lamprey to grow and reach metamorphosis. Instead, each year larval assessments are undertaken by Fisheries and Oceans Canada and the US Fish and Wildlife Service to monitor the presence, relative abundance, and size structure of sea lamprey larvae in Great Lakes tributaries. The larvae within a stream are monitored to determine when they have reached sizes (>100 mm) predicted to result in metamorphosis. Streams are ranked for lampricide treatment based on the abundance of large larvae relative to the costs of treating that particular stream.

## Resistance strategies

Resistance is a microevolutionary process that occurs within a population over multiple generations. We define resistance as a reduction in sea lamprey mortality from lampricides as a consequence of genetic change in the population (Sawicki 1987; Mota-Sanchez et al. 2002; Tabashnik et al. 2014). Genetic change that is heritable across generations is required for an evolved response. Our definition includes two primary types of resistance, physiological and behavioural, each of which could result in reduced effectiveness of the sea lamprey control program (Fig. 1). Our definition does not include epigenetic forms of inheritance that do not involve changes in DNA sequences (Jablonka and Raz 2009), although we recognize that epigenetic forms of resistance would also have consequences for sea lamprey control. Our definition also does not include tolerance or acclimation arising within an individual upon prior exposure to a sublethal concentration of a pesticide, because such changes are not transmitted across multiple generations (Wirgin and Waldman 2004; Hua et al. 2013). These nongenetic responses could increase chances of surviving pesticide treatment, but would be quickly reversible when the selective pressure is removed.

Three prerequisites must be met for resistance to evolve and be detectable. First, selection from the control measure needs to be sufficiently strong to elicit genetic adaptation. Second, phenotypic variation in the population that has a genetic basis must provide some individuals with an increased probability of surviving control measures, with a proportion of the variation being heritable. Third, adequate time to detect the genetic response must pass. The selection pressure needs to be sufficiently high to kill many individuals but not so high that the few survivors cannot find and reproduce with one another (Mota-Sanchez et al. 2002). The initial frequency of individuals with resistant genotypes provides the basis on which selection acts. Resistant genotypes might be present at an initial low level within the founding population (depending on the genetic variation) or arise through mutation or immigration of new individuals. If selection is strong enough and a sufficient number of generations have passed, the frequency of resistant individuals in the population will increase over time, and eventually the presence of resistance will be detectable as a reduction in the mortality from the control measure.

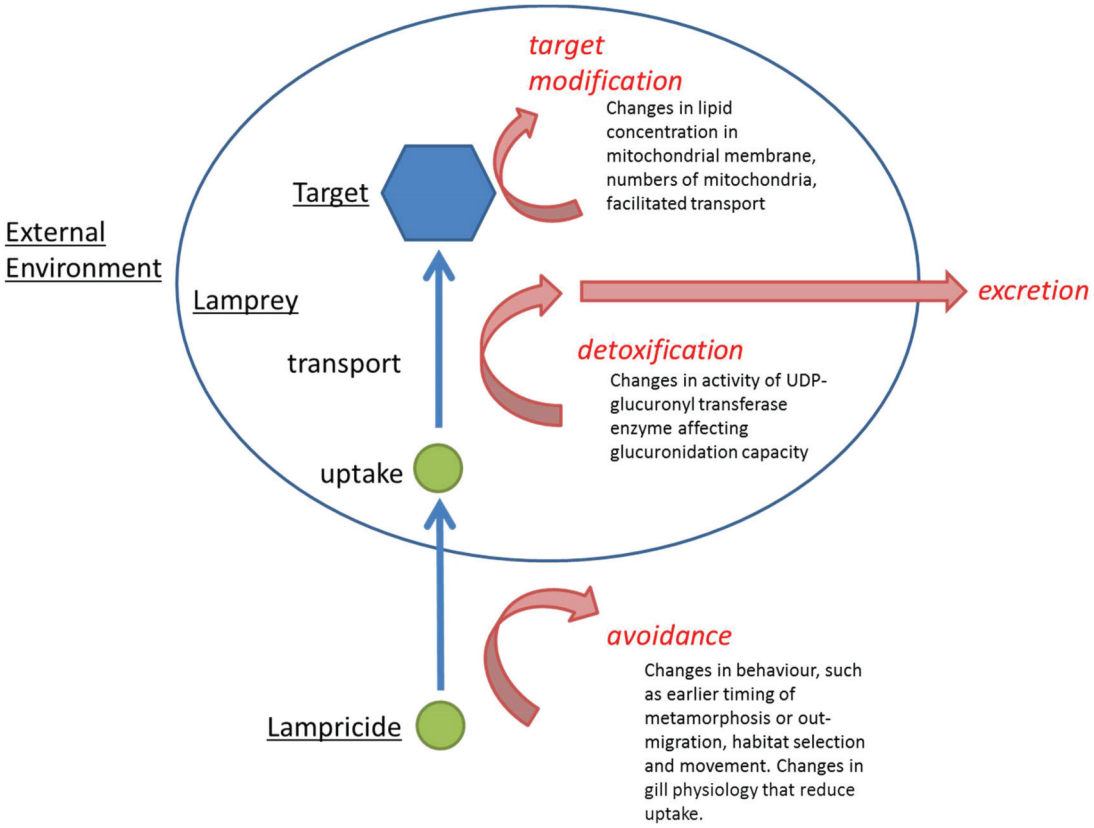
Most documented examples of pesticide or antibiotic resistance are physiological (Williamson et al. 1996; Wright 2007), probably related to the fact that it can be demonstrated in the laboratory by measuring lethal concentrations, such as the $LC_{50}$ or $LC_{99}$ (the concentrations required to kill 50% or 99% of the population, respectively). Behavioural resistance is more difficult to demonstrate because the behaviour of targeted animals is often difficult to study in the field, behavioural studies of pests typically focus on populations rather than individuals, identifying the genetic basis for behaviour can be difficult, and toxicity tests conducted in the laboratory are not usually suitable for studying complex behaviours (Sparks et al. 1989; Ranson et al. 2011; Morales et al. 2013). Both types of resistance strategies can evolve in a population simultaneously, and interactions between behaviour and physiology occur (Sparks et al. 1989).

### Behavioural resistance

As a first line of defense, organisms can evolve behaviours to avoid encounter or reduce their uptake of a pesticide (Sparks et al. 1989; Ranson et al. 2011). One of the few well-documented examples of behavioral resistance occurred in the sheep blowfly (*Lucillia cuprina*) when females evolved the strategy of avoiding laying eggs in the presence of an insecticide aimed at their larvae (Mariath et al. 1990).

Behavioural resistance to lampricides could arise in sea lamprey via the evolution of behaviours or life history strategies that enable sea lamprey to avoid exposure or circumvent death despite exposure to a treatment. Tributaries are typically treated with lampricide on a cyclical basis to coincide with the time it takes larvae to reach their age and size at metamorphosis. This repeated, cyclic nature of lampricide applications could select for earlier age at metamorphosis, thereby increasing the probability of individuals leaving a tributary between treatments. Similarly, lampricide treatment could select for individuals that avoid treatment by out-migrating to lakes before metamorphosis. Normally, out-migration is timed with metamorphosis, but the movement of larvae downstream as they age (Manion and McLain 1971; Manion and Smith 1978; Dawson et al. 2015) or the presence of larvae in lentic areas at the mouths of some tributaries (Fodale et al. 2003) suggests that other variables or behaviours could be involved in out-migration timing. Whether these strategies evolve will depend on balancing any advantages gained against any po-

**Fig. 1.** Examples of potential routes of lampricide resistance evolution in sea lamprey. In susceptible lamprey, lampricide is taken from the external environment into the body (uptake) and transported to cell mitochondria where it uncouples mitochondrial oxidative phosphorylation and inhibits ATP production, resulting in death to the individual. Routes of resistance evolution are indicated in red italics and include *avoidance* of uptake (via behavioural or physiological strategies) or *detoxification*, *excretion*, and *target modification* (via physiological strategies). Example evolutionary changes that could bring about resistance are given in black text. Potential routes and examples given here are not meant to be exhaustive. Figure is modified in part from Despres et al. (2007).




tential costs of these behaviours such as increased predation pressure or reduced growth. The presence of exogenous factors such as temperature and discharge that are potentially involved with triggering downstream movement (Dawson et al. 2015) might also have a bearing on the evolution of behavioural resistance.

Repeated lampricide treatments could also select for larval lamprey that prefer local habitats where lampricide exposure is reduced. These habitats could include stream reaches above locations where treatments are initiated or secondary habitats where it is difficult to achieve lethal concentrations of lampricide, such as side channels, backwaters, and oxbows (Adair and Sullivan 2014*b*). Similarly, treatment could select for changes in the behavioural response to lampricide exposure. Treatment crews observe larval sea lamprey moving into side channels, still-water areas, or onto mudflats upon lampricide exposure, which might offer a selective advantage if these behaviors have a genetic basis. Lampricide-induced selection could potentially also act on burrowing behaviour, although evidence suggests that burrows offer little protection against lampricide (Bills et al. 2003).

Body condition and the timing, amount, and level of feeding activity could also evolve in response to lampricide treatment. Larvae are more susceptible to lampricide in spring than in summer (Applegate et al. 1961; Scholefield et al. 2008), likely a result of differences in body size and glycogen and lipid stores (Wilkie et al. 2014). Large, high-condition sea lamprey suffer less mortality from lampricides, possibly creating a selection gradient that could lead to evolution of life history characteristics over time. In many ways the evolution of life history traits in response to lampricide treatment is similar in concept to the rapid evolution of traits like body size or maturation schedule that have been documented in fish stocks in response to harvest (Jørgensen et al. 2007).

## Physiological resistance

Once bypassing any behavioural means of avoiding or reducing uptake of the chemical, physiological adaptation provides the next opportunity for evolving resistance. Physiological mechanisms that enable uptake rates to be reduced or slowed at the gills could provide a site for selection. Following uptake of the chemical into the body, there are then many additional routes for physiological resistance to evolve (Fig. 1), all resulting in reduced toxicity of the chemical to the organism. In sea lamprey, these routes for physiological resistance to TFM and niclosamide would likely be related to the mode of action of these pesticides, which exert their toxicity by uncoupling mitochondrial oxidative phosphorylation (Niblett and Ballantyne 1976; Birceanu et al. 2011).

Oxidative phosphorylation takes place in the mitochondria of cells and results in the production of adenosine triphosphate (ATP), which provides the energy needed by an organism to function. The process requires oxygen and takes place on the inner mitochondrial membrane, where three of the protein complexes (I, III, IV) that make up the electron transport chain pump protons from the matrix of the mitochondria into the intermembrane space. This creates a gradient for protons to flow back into the matrix. Owing to the low permeability of the inner membrane, the flow of the protons back into the matrix is directed through another protein complex, the ATP synthase. As the protons move down their gradient, energy is released that is used for ATP production.

Chemicals that uncouple oxidative phosphorylation target the inner mitochondrial membrane by either increasing its permeability to protons or by acting as protonophores that shuttle protons back into the mitochondrial matrix (Terada 1990). This reduces the proton gradient for the movement of protons into the mitochondrial matrix via the ATP synthase, lowering ATP production. Eventually, this uncoupling mechanism creates a mismatch between ATP supply and demand, forcing the organism to rely on anaerobic fuels such as glycogen, glucose, or phosphocreatine to generate ATP (Birceanu 2009; Clifford et al. 2012; Birceanu et al. 2014). Once these energy stores are depleted, the organism can no longer meet its energy demands and death ensues (Hollingworth 2001).

Recent evidence suggests that TFM likely uncouples oxidative phosphorylation by acting as a protonophore, reducing ATP production in a concentration-dependent fashion in sea lamprey and some other species such as rainbow trout (*Oncorhynchus mykiss*) (Birceanu et al. 2011). Less is known about the mode of action of niclosamide in sea lamprey, but the weight of evidence suggests that it too uncouples oxidative phosphorylation (Ishak et al. 1970; Nettles et al. 2001; Tao et al. 2014) by acting nonspecifically as a protonophore (Jurgeit et al. 2012).

Several potential routes exist for the evolution of physiological resistance to TFM (and likely also niclosamide), and we describe only a few here. One route is for resistance to develop at the level of the mitochondria. For instance, a change in mitochondrial sensitivity to TFM could evolve via a change in mitochondrial membrane lipid composition (Monteiro et al. 2011) or an increase in the number of mitochondria in a cell, which changes the efficacy of the lampricide. A transporter that facilitates the shuttling of TFM across the mitochondrial membrane could be another site for resistance development, although evidence for the existence of such a transporter for TFM is lacking. Another route of physiological resistance could come about through increased detoxification capacity. Phase II metabolism via glucuronidation of TFM, catalyzed by the enzyme UDP-glucuronyl transferase (UDP-GT), appears particularly important in the detoxification process, resulting in the formation of water-soluble TFM-glucuronide, which is more easily excreted and likely to be inactive as an uncoupler (Lech and Statham 1975; Kane et al. 1994). Sea lamprey have very low glucuronidation capacity compared with some other fishes, which explains their higher sensitivity to TFM (Lech and Statham 1975; Kane et al. 1994). However, genetic variation among individual sea lamprey in their glucuronidation capacity (e.g., stemming from variation in the interaction between UDP-GT and TFM) could provide a site for resistance development. Genomic analyses indicate that sea lamprey express at least two isoforms of the UDP-GT gene (Smith et al. 2013), but further research is needed to better characterize the functional properties of the isoforms in sea lamprey if we are to predict if it is a potential target for resistance selection. Similar arguments also apply to niclosamide, but its detoxification is more complicated, involving not only glucuronidation but also sulfation, in which another sulfate group is added to the compound to facilitate its excretion (Hubert et al. 2005).

Like other pesticides that uncouple mitochondrial oxidative phosphorylation, TFM and niclosamide lack a specific binding site, which could limit the way physiological resistance evolves. Because TFM and niclosamide act as proton shuttles across the inner mitochondrial membrane, rather than targeting a specific protein (e.g., a receptor), it is difficult to envision how naturally selected changes could be induced at this level. A mutation at the binding site of a toxic chemical, a frequent cause of physiological resistance evolution in agricultural pests and bacteria (ffrench-Constant et al. 2004; Wright 2007), therefore would not be a likely mode of resistance development to lampricide.

Despite the lack of a binding site, however, resistance to mitochondrial uncoupling pesticides has occurred in agricultural systems (e.g., Van Leeuwen et al. 2006; Ahmad and Arif 2009; Leroux and Walker 2013). In one example, resistance was related to increased activity of a transmembrane transporter (Leroux and Walker 2013), and in another example, resistance appeared related to enhanced activity of the esterase enzyme (Van Leeuwen et al. 2006). Organisms have used a variety of mechanisms to evolve resistance to chemicals present in their environment, including strategies that do not involve binding site mutations (Posthuma and Vanstraalen 1993; Despres et al. 2007).

## Examples of resistance from other systems

While arthropods have a long history of evolving mechanisms to defeat toxins (Mota-Sanchez et al. 2002), fewer examples of vertebrate pests developing resistance to chemical control have been documented. The best known vertebrate example is that of rodents evolving resistance to rodenticides that inhibit blood coagulation. Application of the anticoagulating rodenticides (most notably warfarin) began in the early 1950s, with the first published evidence of resistance in 1958 (Boyle 1960) and with documented cases of resistance arising in numerous countries since (Pelz et al. 2005). Another example of resistance in vertebrate pests is that of European rabbits (*Oryctolagus cuniculus*) developing resistance to Compound 1080, a pesticide used heavily in baited traps in Australia and New Zealand since the 1950s to control non-native mammals. The first signs of reduced bait effectiveness in rabbits arose in the 1970s (Oliver et al. 1982), with follow-up toxicity tests confirming resistance that was related to the population's history of exposure in the field (Twigg et al. 2002).

Some vertebrates, particularly fishes, have developed resistance to chemical toxicants present in their environment (Wirgin and Waldman 2004). Atlantic tomcod (*Microgadus tomcod*) in the Hudson River, New York, offer one of the most compelling examples of a fish population rapidly evolving resistance to chemicals. When embryonic offspring of Atlantic tomcod from more pristine environments are exposed to coplanar polychlorinated biphenyls (PCBs) or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), they typically develop a range of developmental abnormalities that likely result from impaired heart structure and function. However, the offspring of tomcod from the Hudson River, where release of industrial pollutants began in the late 1940s, do not show these responses and instead demonstrate reduced embryonic mortality to PCBs and TCDD and decreased expression of the cytochrome P4501A (CYP1A) gene. Results from controlled laboratory crosses revealed that the resistance was heritable across at least the $F_2$ generation (Wirgin et al. 2011). The genetic basis of resistance was shown to arise from a single two amino acid deletion in the aryl hydrocarbon receptor 2 (AHR2), a transcription factor that mediates toxicity and activation of numerous genes in the AHR2 pathway, including CYP1A. A standing variant of AHR2 was nearly fixed in the Hudson River population and almost absent elsewhere (Wirgin et al. 2011).

Several factors may have contributed to resistance evolution in Hudson River tomcod. The selective pressure experienced by the population was strong due to the chronic exposure of critical early life stages to high concentrations of these sediment-borne contaminants. Also, although the population was panmictic within the Hudson River, they were reproductively isolated from other sensitive populations, preventing reintroduction of sensitive alleles via gene flow and facilitating local adaptation (Wirgin et al. 2011). Finally, the fact that resistance was attributable to a single standing variant in one gene likely accelerated the evolutionary process. The year when resistance would have been first detectable is not known because the population was not tracked through time, so we only know that it occurred sometime within the 70 years after contamination began. The generation time of tomcod (approximately 1–2 years) is less than that of sea lamprey, but this example does demonstrate that fish populations are capable of rapidly evolving resistance to toxic chemicals in the environment.

## Meeting the prerequisites for resistance evolution in sea lamprey

Resistance has been observed to evolve within a matter of weeks, months, years, or decades depending on the organism in question and the strength and mechanism of selection (Palumbi 2001). Below, we evaluate whether the selective environment has been conducive for resistance evolution by estimating the number of generations of sea lamprey that have been exposed to lampricides and the strength of the selective pressure from TFM.

### Has there been enough time for evolutionary change to be observed?

Assuming a generation time of 5 years, Great Lakes sea lamprey have been exposed to lampricides for between 6 and 11.6 generations (Table 1), placing them within the range of other fish populations where evolution has been documented (Table 2). A 5-year generation time for sea lamprey is an approximation based on observations of time between recruitment and metamorphosis. The age at metamorphosis typically ranges from about 3 to 5 years, depending on the lake (generally, ages 3–4 for Michigan, Erie, and Ontario; age 4 for Huron; age 5 for Superior; M. Steeves, personal observation), followed by a parasitic juvenile phase of 12–18 months prior to spawning (Bergstedt and Swink 1995). We therefore chose a generation time toward the lower end of the range because we wanted to evaluate the possibility that enough time has passed for resistance evolution. Even a more conservative estimate for generation time in Lake Superior of 7 years would mean exposure to lampricides for 8.3 generations, still well within the range reported for rapid genetic change in other fish populations (Table 2).

There is growing awareness of the rapid pace of evolution that is possible in vertebrate populations, most often as a result of human-induced selective pressures (Hendry et al. 2017). In fish populations, where generation times are comparable to sea lamprey, evolution has occurred on surprisingly short time scales in response to selection from a variety of sources, including harvesting, toxins, predation, and introduction into novel environments (Table 2). Based on a review of published accounts of evolution in fishes, we see that evolution has been documented in as few as three generations and spanning as many as 140 generations (Table 2). The generation times of fish in these studies ranged from months (e.g., 2–3 months in guppies (*Poecilia reticulata*); van Wijk et al. 2013) to several years (e.g., about 4 years in sockeye salmon (*Oncorhynchus nerka*); Hendry et al. 1998). The types of selective pressure were diverse, ranging from strong harvesting imposed in a laboratory, such as 90% harvest rate in Atlantic silverside (*Menidia menidia*) (Conover and Munch 2002) to natural selection acting on a population introduced to a novel environment, as observed for mosquitofish (*Gambusia affinis*) (Stockwell and Weeks 1999).

Rapid changes in fish life history attributed to evolution have also occurred in several wild stocks experiencing selective pressure from commercial fisheries (Jørgensen et al. 2007). The generation time of these commercial species is relatively long, for example, close to a decade for Atlantic cod (*Gadus morhua*) (Eikeset et al. 2013). Trends in maturation-related traits attributed to fisheries-induced evolution have been reported in as few as 1.8 to 12 generations (Devine et al. 2012), although identifying the genetic contribution to the changes is difficult (Hansen et al. 2012).

### What is the selective pressure from lampricides?

At this time, we do not have a direct measure of the strength of selection from lampricides on sea lamprey in the Great Lakes. Various approaches have been used to estimate selection pressure (Kinnison and Hendry 2001; Siepielski et al. 2009; Laugen et al. 2014), generally requiring data on responses to selection for a particular trait, behaviour, or mutation. The selection gradient is a measure of direct selection on a trait and can be measured as the covariation between the trait and fitness (Lande and Arnold 1983). In the case of sea lamprey resistance, where the source of selection is direct mortality from lampricides, the selection gradient is generally expected to increase as mortality increases because there is predicted to be a larger difference between the genotypes of those individuals that survive (the resistance genotypes) than those that are susceptible to the pesticide. The selection gradient together with the genetic variation of the trait in question will affect the magnitude or rate of evolution. For sea lamprey resistance, we lack a specific trait, mechanism, mutation, or behaviour for which to estimate selection gradients. For the purposes of this paper and until such data are available and analyzed, we use the mortality rate from lampricides as an indicator of the strength of selection, but it must be emphasized that this is not a direct measure.

To quantify lampricide-induced mortality, we estimated the fraction of the sea lamprey population killed from lampricides since implementation of the control program. Making use of an operating model and existing data, we estimated that lampricide-induced mortality in 2012 ranged from a high of 90% in Lakes Huron and Ontario to a low of 82% in Lake Superior (Table 3; Appendix A). For Lake Superior, the mortality estimate derived from the operating model (82%) was only slightly lower than an estimate (89%) obtained from control program data on larval abundances (Appendix A). Furthermore, these estimates are close to previous suggestions that sea lamprey abundances in the Upper Great Lakes were reduced by 90% of precontrol levels (Smith and Tibbles 1980; Heinrich et al. 2003), although factors other than TFM application likely contributed to the declines in abundance (M. Siefkes, personal communication). From these various estimates, we conclude that the strength of selection from lampricides could have been substantial. To provide some context, these mortality rates are comparable to those used in harvest selection experiments on Atlantic silverside where life history evolution arose within four generations (Conover and Munch 2002).

Estimates of lampricide-induced mortality might underestimate the overall strength of selection from lampricides if there is selection in response to sublethal effects. Lampricide exposure has the potential to cause both mortality and sublethal effects in the sea lamprey that survive a treatment (Clifford et al. 2012). Many species exhibit sublethal responses to chemicals if exposed to concentrations insufficient to cause mortality (Relyea and Hoverman 2006). Examples of sublethal effects include changes in physiology, immune response, behaviour, and reproductive success (Weis et al. 2001; Relyea and Hoverman 2006). Sublethal responses to TFM could impact fitness and, if related to genetic variation among individuals, could evolve in response to lampricide treatment. In future, more direct measures of the strength of selection would enable more accurate predictions as to the likelihood and rate of resistance evolution in sea lamprey.

## Evidence for resistance in sea lamprey

Several aspects of sea lamprey biology and control offer clues about the likelihood of resistance evolution (Table 4). However, weighing these factors against the potential evolutionary costs of evolving strategies to evade control is challenging. Ultimately, experimental data are required to demonstrate that resistance has evolved or that the number of resistant individuals in the population is increasing.

Evidence for physiological resistance can be obtained by tracking changes in the lethal concentrations of a pesticide required to kill fixed percentages of test organisms. The prediction is that the $LC_{50}$ would increase if resistance had evolved. Taking this approach, we analyzed the outcomes of historical TFM toxicity tests that have regularly been conducted on sea lamprey larvae since 1956 (Appendix B). Over the 57-year period of toxicity testing, no

**Table 2.** Published examples of studies in which evolution was documented for fish populations exposed to selection.

| Citation | Species | Trait | Type of study | Agent of selection | No. of generations[a] |
|---|---|---|---|---|---|
| van Wijk et al. 2013 | Trinidadian guppy (*Poeciala reticulata*) | Male body size | Artificial selection in laboratory with genetic analysis | Harvest | 3 |
| Gordon et al. 2015 | Trinidadian guppy (*P. reticulata*) | Male color (secondary sex characteristic) | Introduction experiment, including monitoring, genetic analysis, and common garden analysis | Introduction to novel environments differing in predation | 3 |
| Conover and Munch 2002 | Atlantic silverside (*Menidia menidia*) | Body size | Artificial selection in laboratory | Harvest | 4 |
| Breckels and Neff 2014 | Trinidadian guppy (*P. reticulata*) | Sperm length | Artificial selection in laboratory | Temperature | 4 |
| Reznick et al. 1990; Reznick and Bryga 1987 | Trinidadian guppy (*P. reticulata*) | Maturity | Translocation experiment of wild populations | Introduction to novel environments differing in predation | 7 |
| Hendry et al. 1998 | Sockeye salmon (*Oncorhynchus nerka*) | Developmental rate (time to hatch; time to emerge) | Common garden experiments of wild populations | Introduction to novel environment | 14 |
| Aykanat et al. 2011 | Rainbow trout (*Oncorhynchus mykiss*) | Osmoregulatory genes | Common garden experiments and genetic analysis of wild populations | Introduction to novel environment (fresh water) | 14 |
| Du et al. 2016; Nacci et al. 1999, 2010; Reid et al. 2016 | Killifish (*Fundulus heteroclitus*) | Hepatocyte oxidative phosphorylation metabolism (PCB resistance) | Genetic and common garden experiments of wild populations | PCBs | 15 |
| Kavanagh et al. 2010 | Arctic grayling (*Thymallus thymallus*) | Developmental traits (growth rate; mass development rate; conversion efficiency) | Common garden experiment and genetic analysis of wild populations | Introduction to novel environment (cold versus warm) | 22 |
| Pearse et al. 2009; Phillis et al. 2016 | Rainbow trout (*O. mykiss*) | Size-at-age and threshold size for migration tactic (evolution of residency) | Common garden and genetic analysis of wild populations | Translocation above a migration barrier | 25 |
| Kinnison et al. 1998*a*, 1998*b* | Chinook salmon (*Oncorhynchus tshawytscha*) | Time to hatch; growth rate | Common garden experiment of wild populations | Introduction to novel environment | 26 |
| Westley et al. 2012 | Brown trout (*Salmo trutta*) | Juvenile survival | Common garden and in situ reciprocal transplant | Introduction to novel environment | 37 |
| Hubert et al. 2016 | Common carp (*Cyprinus carpio*) | Scale cover | Breeding experiment and genetic analysis of wild and hatchery fish | Introduction to novel environment | 40 |
| Wirgin et al. 2011 | Atlantic tomcod (*Microgadus tomcod*) | PCB resistance | Common garden and genetic analysis of wild populations | PCBs | 50 |
| Lescak et al. 2015 | Threespine stickleback (*Gasterosteus aculeatus*) | Morphology (mainly lateral plate number) | Genetic analysis of wild populations | Habitat shift | 50 |
| Stockwell and Weeks 1999 | Mosquitofish (*Gambusia affinis*) | Size at maturity; fat content | Common garden experiment of wild populations | Introduction to novel environment | 110 |
| Walsh et al. 2016; Walsh and Reznick 2008, 2011 | Trinidadian killifish (*Rivulus hartii*) | Brain size; size and age at maturity; reproductive investment | Common garden experiment of natural populations | Predation | 114 |
| Stearns 1983 | Mosquitofish (*G. affinis*) | Age and length at maturity; offspring size (mass) | Common garden experiment of wild populations | Introduction to novel environment | 140 |

**Note:** Examples were restricted to those with strong evidence for evolution as opposed to the sole result of phenotypic plasticity; this included studies where genetic assessment or common garden experiments were conducted to provide evidence of evolution. Note that the number of generations that had passed by the time the study or analysis was done could be greater than the number of generations in which an evolutionary response could have been detected. The list of examples is not exhaustive.

[a]Maximum value in cases where a range of generation times were given.

**Table 3.** Estimated reductions in parasitic sea lamprey recruitment attributable to lampricide control.

| Lake | No. of adults | Budget | Median no. of parasites with no control | Median no. of parasites with control | Percentage killed as a result of control |
|---|---|---|---|---|---|
| Superior | 760 000 | $1 010 000 | 2 091 954 | 370 767 | 82% |
| Michigan | 1 850 000 | $2 030 000 | 3 084 211 | 475 999 | 85% |
| Huron | 1 910 000 | $2 280 000 | 5 394 502 | 521 587 | 90% |
| Erie | 154 000 | $270 000 | 199 007 | 24 796 | 88% |
| Ontario | 311 000 | $680 000 | 823 147 | 78 474 | 90% |

**Note:** Mortality rates were based on a model (Jones et al. 2009) that simulates sea lamprey population dynamics and management at the level of an entire Great Lake and calibrates lake-wide abundance to correspond with recent adult assessments and recent lampricide control expenditures. Model incorporates uncertainty in stream-level lampricide effectiveness and stream-level assessment and recruitment stochasticity. Results are for 1000 simulations.

**Table 4.** Summary of Great Lakes sea lamprey life history and control program attributes relevant to the development of resistance to lampricides.

| Life history or control program attribute | What it might mean for resistance |
|---|---|
| Generation time of about 5 years | This limits the number of generations of sea lamprey that have been exposed to lampricides and slows the rate of resistance development relative to most other taxa where resistance has been observed. However, this does not prevent the possibility of rapid evolution, as numerous studies of fish populations have demonstrated. |
| Absence of natal homing | Sea lamprey born from different tributaries mix during reproduction, potentially diluting selection because mating occurs between larvae exposed to TFM and those not exposed. Similarly, random mating between resistant and nonresistant genotypes might slow the evolution of resistance. Little to no local adaptation would also reduce the chances of a population of resistant (or susceptible) individuals immigrating and spreading resistant (or susceptible) genotypes. Alternatively, this mixing might mean resistance will spread rapidly if it does arise. |
| Invasion history | The source population(s) of sea lamprey may have possessed limited genetic variability, potentially limiting the number of resistant genotypes present in the founding population. However, some research has suggested that rapid rates of adaptation are still possible in invading populations even though genetic diversity might be low (Dlugosch and Parker 2008). |
| Mortality rate from lampricides | Lampricide-induced mortality rate estimates are high (>80%), increasing the selective pressure and thus increasing the chances of resistance evolution. |
| High fecundity | Based on studies of pest resistance in arthropods, high fecundity increases the chances of resistance evolution because the survival and mating of just a few resistant individuals can potentially produce large numbers of resistant offspring. Furthermore, high fecundity increases the chances of novel mutations conferring resistance. |
| Residual sea lamprey | The many ways in which sea lamprey could survive or avoid treatment will weaken selection and reduce the likelihood of physiological resistance — for example, lamprey could potentially survive treatment not only by having a resistant genotype, but also because not all streams or stream reaches are treated in a given year (i.e., subject to variable selection). Other examples by which residual sea lamprey might survive include residing in backwaters and eddies where lethal concentrations of TFM are more difficult to achieve or where treatment is not applied because densities are relatively low. However, the survival of these residual sea lamprey could increase the chances of behavioural resistance evolution and raise the overall probability of a mutation arising by increasing the pathways for resistance evolution. |
| Reliance on 3-trifluoromethyl-4-nitrophenol (TFM) as the primary method of sea lamprey control | Although integrated pest management is a goal of the sea lamprey control program, chemical treatment of streams with TFM is the main method of control used. This increases the chances of resistance development. |
| Cyclical nature of treatment | This could increase the chances of certain types of behavioural resistance, for example earlier transformation or out-migration. |



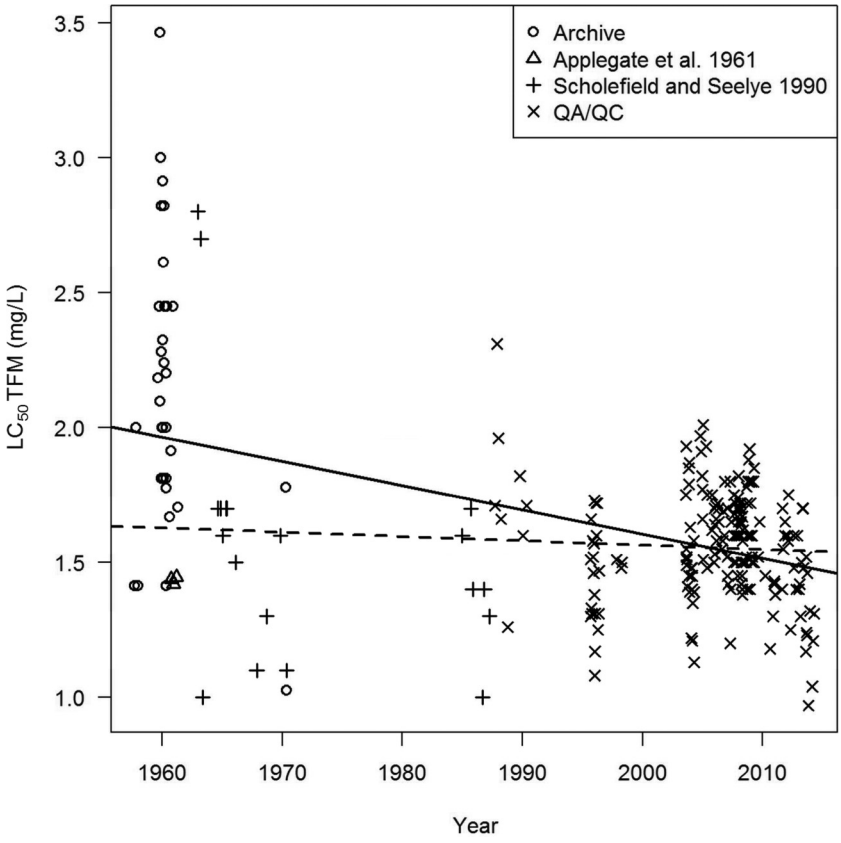
increase in the TFM concentration at which 50% of the sample lamprey died (the $LC_{50}$ over 19–24 h; Fig. 2) was detected. An earlier analysis covering a shorter period of time (30 years) also found no evidence of changes in TFM toxicity in larval sea lamprey (Scholefield and Seelye 1990).

Our findings of no change in the toxicity to TFM over time should be interpreted cautiously when drawing conclusions about the evidence for resistance. Our analyses relied on historical data that were collected for other purposes, not with the intent of specifically investigating the evolution of resistance (see Appendix B for more details about the tests). Sample sizes were low for some years, and key variables known to influence toxicity (e.g., pH, source stream of the larvae, temperature, time of source larvae collection) were not always measured or controlled. Furthermore, the cause of higher estimates of $LC_{50}$ values from the archival data relative to the other sources (Fig. 2) is unknown and could indicate differences in testing conditions. Appropriately designed experiments are needed to rule out the possibility of lampricide resistance in sea lamprey.

## Looking to the future: strategies to reduce the risk of resistance

Management actions aimed at reducing resistance to pesticides include (*i*) diversifying control measures, (*ii*) allowing survival or immigration of susceptible individuals, (*iii*) monitoring to detect

**Fig. 2.** Results of historical toxicity tests showing concentrations of 3-trifluoromethyl-4-nitrophenol (TFM) that killed 50% ($LC_{50}$) of larval sea lamprey. Results are for static toxicity tests run for 19–24 h in Lake Huron water conducted at the Hammond Bay Biological Station. Data were from archival records, historical reports (Applegate et al. 1961; Scholefield and Seelye 1990), and quality assurance tests (QA–QC) of TFM supplies (Adair and Sullivan 2014*b*). Linear regression is shown fit to all data (solid line) and to all but the archival data (dashed line). A small random offset was added to sample from the same year to distinguish overlapping points. The estimated slope for $LC_{50}$ from all data was −0.009 (95% confidence interval: −0.011 to −0.007). Without the archival data, the $LC_{50}$ slope was −0.002, (95% confidence interval: −0.004 to 0.001). Six outliers ($LC_{50}$ > 3) from the archival records were removed. As these high values occurred early in the time series, their removal does not impact the sign of the slope.






resistance, and (*iv*) modeling to predict resistance development (Mota-Sanchez et al. 2002). A few of these strategies could be integrated into current sea lamprey control program procedures.

Diversifying control measures using integrated pest management (IPM) could be a key tool to reduce the likelihood of resistance evolution in sea lamprey. IPM includes using alternative control measures to reduce the ecological costs of chemical pesticides (reviewed in Kogan 1998); these alternative controls often involve behavioural manipulation of pests such as push–pull strategies (Cook et al. 2007) or biological control strategies (Lacey et al. 2001). IPM has been adopted in many agricultural systems to reduce reliance on chemical pesticides and in turn reduce contaminant levels in the environment, improve control, and reduce the incidence of resistance (Kogan 1998). The expectation is that it would be rare for an individual in a pest population to possess resistance genes for multiple control tactics that combine different aspects of an organism's life history, behaviour, or physiology. The selective environment becomes more heterogeneous in the presence of multiple control tactics, delaying the evolution of resistance (Bourguet et al. 2013).

As a concept, IPM has already been embraced by the GLFC and the sea lamprey control program (Sawyer 1980; Christie and Goddard 2003; GLFC 2011). The GLFC first endorsed IPM for sea lamprey control during the 1970s and adopted it officially as part of its policy in the 1980s (Sawyer 1980; Christie and Goddard 2003; Siefkes et al. 2013). This paved the way to exploring the development of alternative control measures, including barriers, trapping, pheromones, and repellants (Siefkes et al. 2013). Besides barriers, however, these alternative control tactics are still under development and do not contribute to the current suppression of sea lamprey. Barriers to migration do limit sea lamprey access to suitable spawning and nursery habitats, but are not present on all streams, rely in part on a network of deteriorating structures constructed for purposes other than sea lamprey control, and face constant pressure to restore watershed connectivity through barrier removal (Lavis et al. 2003*a*; McLaughlin et al. 2013). Thus, although the development of lampricide alternatives remains a top priority for the GLFC (Sawyer 1980; Christie and Goddard 2003; GLFC 2011), the ecology and economy of the Great Lakes fisheries currently depends on TFM to control sea lamprey.

This reliance on a single chemical to control a pest increases the chances of resistance development. Even considering that niclosamide is sometimes used, it is believed to have a similar mode of action. The use of an alternative lampricide that exerts its toxicity through a different mechanism, if discovered, could slow resistance if used alongside TFM. For this reason, the application of two or more toxins with different modes of action has been used to reduce resistance in insect pests (Comins 1986; Roush and Daly 1990; Carrière et al. 2016). The same logic applies when combining chemical pesticides with other control tactics such as barriers — the use of multiple control tactics, each exploiting a different aspect of sea lamprey biology, will reduce the likelihood of TFM

resistance because selection would favor individuals based on a different suite of traits and underlying genotypes. In effect, selection for chemical resistance would be diluted in the presence of effective alternative control tactics.

Research on pheromones used by sea lamprey for reproduction and migration (Li et al. 2002; Meckley et al. 2014; Buchinger et al. 2015) and the sequencing of the sea lamprey genome (Smith et al. 2013) could pave the way for the development of alternative control techniques. Next generation (i.e., new) lampricides could be discovered that have a different mode of action or are more selective, environmentally friendly, and cost effective. For example, identifying circumstances under which a gene is expressed could be used to develop a lampricide that exploits a unique aspect of sea lamprey development (Buchinger et al. 2015; McCauley et al. 2015). In this respect, greater incorporation of high throughput methods, such as RNA sequencing to generate transcriptomes of potential target tissues or organs, could be very fruitful. An emerging genetic approach to control undesirable species and reverse resistance is the engineered gene drive method based on the CRISPR-Cas9 technology, a gene-editing technology using guided RNA that can target and modify DNA and RNA sequences (Champer et al. 2016). The gene drive elements are inherited by offspring and result in the spreading of sterility within the population.

Design and implementation of effective barriers, potentially in association with the use of pheromones (Johnson et al. 2009; Buchinger et al. 2015), offers another potential option for reducing resistance to TFM. Effective barriers have the potential to alter which sea lamprey individuals successfully reproduce, thereby altering the fitness landscape and potentially mitigating selection for pesticide resistance. When upstream-migrating sea lamprey encounter a barrier, several responses have been documented, including swimming around to find ways to circumvent the barrier or to evade capture within traps, fallback and spawning further downstream, or leaving the tributary to find an alternative spawning site (e.g., Bravener and McLaughlin 2013; McLean et al. 2015; Holbrook et al. 2016). These responses require time and energy that could provide a fitness advantage to individuals with certain heritable characteristics; as with other alternative control techniques, it becomes increasingly unlikely that individuals would have the suite of genetic characteristics that confer advantages in an environment with both barriers and chemical treatment.

As mentioned above, barriers do contribute to control of sea lamprey, but there is growing pressure to remove them in many locations to restore connectivity and reduce habitat fragmentation for other organisms (McLaughlin et al. 2013). Often interests conflict between keeping (or repairing) a barrier to prevent sea lamprey from accessing upstream spawning habitat versus removing a barrier to improve accessibility to upstream habitat for desirable fishes. The management decisions in these cases are not straightforward. Furthermore, barriers are not present on every stream with sea lamprey spawning habitat, and many of those that exist are in disrepair. Thus, there is scope for increasing the effectiveness of barriers as a control tactic. Improvements to barrier design that limit sea lamprey passage but permit movement of other fish species (Sherburne and Reinhardt 2016) could provide an alternative control measure used to slow TFM resistance that also serves additional management objectives.

Facilitating the survival and emigration of susceptible individuals is another strategy used to reduce pesticide resistance. This strategy is often employed in agricultural systems to preserve susceptible genes in the population by creating refuges where treatment is not applied, but permitting some gene flow between treated and untreated areas (Gould 1998). At first glance, current control of sea lamprey might appear to be a type of refuge strategy because many stream reaches are untreated. However, the annual high degree of reproductive mixing of adults makes sea lamprey control different from a true refuge strategy. The absence of natal homing weakens the formation of discrete stocks, making it difficult to have refuge populations that have never been exposed to lampricides (i.e., because unexposed individuals would mate with exposed individuals at a high rate). A true refuge strategy would require a higher level of reproductive isolation over a number of years between the refuge population and the populations that are exposed — this is not the case with sea lamprey where the reproductive mixing reduces the reproductive isolation of unexposed individuals. However, although different from a refuge strategy in the strict spatial sense, current sea lamprey control does provide opportunities for sea lamprey individuals to survive and reproduce even if they might be susceptible to TFM if exposed. Furthermore, the variety of ways a sea lamprey could evade mortality from TFM in a given year might weaken the strength of selection for any one type of physiological resistance (Table 3; see section on Behavioral resistance), although it could also increase the overall odds of a mutation developing or promote behavioural resistance evolution. Looking to the future, there might be ways of further taking advantage of those sea lamprey already surviving despite possessing susceptible genes. A more direct type of refuge strategy for sea lamprey could include artificially supplementing the wild population with individuals from a population that has not been exposed to lampricides (e.g., an ancestral source population or a population maintained in the laboratory). However, a refuge approach would require careful consideration and weighing of the potential risks associated with increasing the number of spawning adults and enhancing genetic variation in the population versus the benefits of potentially reducing the potential for resistance.

Monitoring for resistance is an integral component to resistance prevention. This is the case whether lampricide resistance arises slowly, with time to act once initial low numbers of resistant individuals are detected, or spreads quickly, with less opportunity but more urgency to modify operations. In insect pests, the presence of low numbers of resistant individuals provides a warning sign that resistance could become a serious problem if strong selection continues. Early detection has proven to be important in agricultural systems because it is often too late to implement strategies for managing resistance once pesticide failure occurs (Mota-Sanchez et al. 2002; Barres et al. 2016).

Routine post-treatment larval assessments already conducted could be used or augmented for the purpose of monitoring for resistance. For example, changes in residual larvae abundances or life history could be tracked and studied. In addition, the control program should develop an annual, standardized toxicity test specifically designed to monitor physiological resistance development. This test could be integrated into existing procedures to conduct quality assurance of new lampricide batches. Residual lamprey that survive treatment in the field or in a laboratory toxicity test could be collected, stored, and later used to investigate mechanisms of resistance development. One potential test for resistance would be a comparison of toxicity between sea lamprey from the Great Lakes and those from a source population outside the Great Lakes basin. Monitoring for physiological resistance would be more straightforward than monitoring for behavioural resistance because of the added challenge of replicating field conditions and inducing natural behavioural responses in a laboratory environment.

Screening for resistance alleles, in particular before resistance becomes a problem, can provide valuable information. The initial frequency of resistant alleles in a population critically affects the risk of resistance development, as does the mode of inheritance (i.e., whether the trait is recessive or dominant; Gould et al. 1997). Laboratory crosses where subsequent generations were followed in the lab (Gould et al. 1997) and DNA-based screening to identify recessive alleles (Gahan et al. 2001) have been used for monitoring the critical early phases of resistance development in insects. Furthermore, data on the frequency of resistant alleles in a population are critical for models that themselves can be a useful tool for

predicting, understanding, and managing resistance (Bourguet et al. 2010; Renton et al. 2014).

## Beyond sea lamprey: implications for invasive species control and ecosystem management

The possibility of sea lamprey developing resistance to chemical control is relevant more broadly in the context of invasive species management. Invasive species possess the capacity to evolve a diversity of physiological and behavioural strategies in response to control and management measures. Many of these strategies better known for bacteria or insects appear relevant for other taxa, including vertebrates. For example, the routes of potential resistance evolution we outlined for sea lamprey (Fig. 1) have basic similarities to the mechanisms insects have naturally evolved to resist plant toxins (Despres et al. 2007).

In many cases, the methods used to control invasive species are likely to cause the strong, directional selection needed to facilitate resistance evolution. Assessing the magnitude of selection from control poses challenges because of compensatory demographic processes and because it can be difficult to tease apart environmental effects when examining phenotypic data collected in the wild. However, in the fight against an invasive species, target mortality rates from a given control measure could be high and when implemented could produce selection differentials comparable to those that have caused rapid evolution in experimental settings and in simulations (Conover and Munch 2002; Reznick and Ghalambor 2005; Dunlop et al. 2009).

Awareness that evolution in response to human-induced selection pressure can occur quickly in animal populations is becoming more common. We did not find any evidence that physiological resistance to TFM has developed thus far in sea lamprey, possibly because not enough generations have passed or for some other reason related to sea lamprey biology or control (Table 3). However, numerous other aquatic invasive species have shorter generation times, increasing the speed over which evolution could arise. For example, two prominent invasive fish species, round goby (*Neogobius melanostomus*) and bighead carp (*Hypophthalmichthys nobilis*), can mature at ages of 1–2 years and 2–4 years, respectively (Gutowsky and Fox 2012; Cooke 2016). Furthermore, rapid evolution can occur within time scales relevant to management even in species with later ages of maturity, including fishes (Fraser 2013).

The perception that fish populations will not evolve over time scales relevant to management is outdated. The development of resistance is just one example of how the speed and capacity of populations to evolve is integral to many major global challenges (Carroll et al. 2014). Species targeted for control or harvest can evolve strategies for evasion more quickly than desired, while species targeted for conservation efforts may evolve too slowly in response to climate change and other stressors. Control programs like that for sea lamprey in the Great Lakes can serve as quasi-experiments that help us understand how the risks of resistance evolution extend to a broader range of taxa, ecological circumstances, and management challenges.

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## Appendix A. Sea lamprey mortality from lampricide treatment

We used two approaches to estimate the mortality rate induced by lampricide treatments. In the absence of direct estimates of selection differentials, we use these estimates as an indication of the potential strength of selection from lampricides. Note that these estimates are different from measurements of treatment effectiveness on a tributary. Lampricide actions are taken at the scale of individual streams, but the sea lamprey they target are from a lake-wide population that does not demonstrate natal homing. Measuring the strength of selection from lampricides requires integrating estimates of effectiveness across all potential lotic and lentic sources of juveniles to a lake.

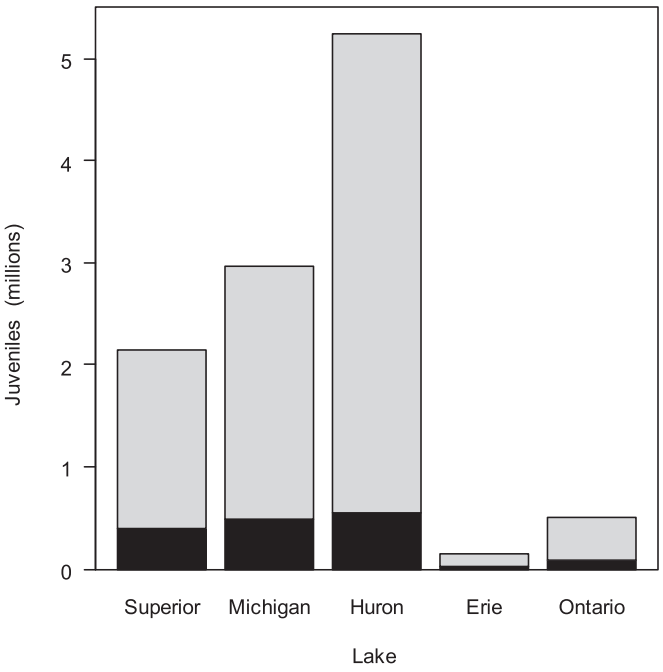
The first approach involves an empirical approximation of lampricide mortality from data that control agents use to decide where to apply treatments. Potential sea lamprey source streams are surveyed regularly for the presence of larvae potentially large enough to metamorphose into parasitic juveniles within a year. These survey data are used to rank streams for lampricide treatment each year, based on the estimated abundance of large larvae relative to the costs of treating that stream. These data are maintained in a database that can also be used to quantify the expected effectiveness of a multiyear lampricide treatment strategy, based on assumptions about the effectiveness of individual stream treatments. Focusing solely on Lake Superior, we used median estimates of larval abundance by stream for 1996 to 2012, and then used the treatment schedule in 2012–2014 and that proposed for 2015, to apply TFM mortality to the individual streams. Several assumptions were made to derive estimates of mortality from the control data: (*i*) larval sea lamprey remain in Lake Superior streams for at least 4 years, (*ii*) 98% of the 2012 year class of larvae present in Lake Superior streams known to contain larval sea lamprey would have been exposed to lampricide treatments, and (*iii*) average TFM treatment effectiveness for a stream was 90%. From this, we estimated the mortality rate for the 2012 year class as 89%.

There are several uncertainties associated with this first approach for estimating mortality from lampricides. The assumptions of 90% treatment effectiveness and 98% exposure to lampricides are simplifications that likely are not always reflective of true control outcomes, thus contributing error to the mortality estimates. Another assumption contributing error is that all source populations of sea lamprey have been assessed.

Given the uncertainties of the first approach, we also estimated mortality rates using a second approach based on an existing operating model (Jones et al. 2009). The model simulates sea lamprey population dynamics and management at the level of an entire Great Lake and calibrates lake-wide abundance to correspond with recent adult assessments and recent lampricide control expenditures. The model also explicitly incorporates uncertainty in stream-level lampricide effectiveness and in stream-level assessment, as well as stochasticity in recruitment dynamics. Thus, the model addresses several of the uncertainties associated with the first approach for estimating mortality.

Simulations using the sea lamprey operating model were used to quantify mortality experienced by a lake-wide cohort of sea lamprey due to lampricide treatment (these are the mortality rates presented in the main text of the paper). First, we calibrated the model for each Great Lake by adjusting larval natural mortality rates such that recent observed adult sea lamprey abundance levels were forecasted, on average, when recent levels of lampricide control were applied (cf. Irwin et al. 2012). Second, we ran simulations for each lake with no lampricide control applied to estimate population abundances associated with no control. Third, we fixed abundance of adults at these "no control" abundances and estimated abundance of parasitic juvenile sea lamprey that would be produced, on average, in the absence of control (averaged across stochastic variation in recruitment dynamics). Fourth, we repeated these simulations with fixed adult abundance but with recent levels of lampricide control and again estimated average abundance of parasitic juvenile sea lamprey produced (in this case averaged across uncertainty in recruitment, assessment, and lampricide effectiveness). Finally, we calculated the ratio of forecasted juvenile abundance with control to that with no control and subtracted this ratio from 1 to obtain our estimate of lake-wide lampricide-induced mortality. The resulting lake-wide estimate of mortality ranged from 82% to 90% (Table 3 main text; Fig. A1).

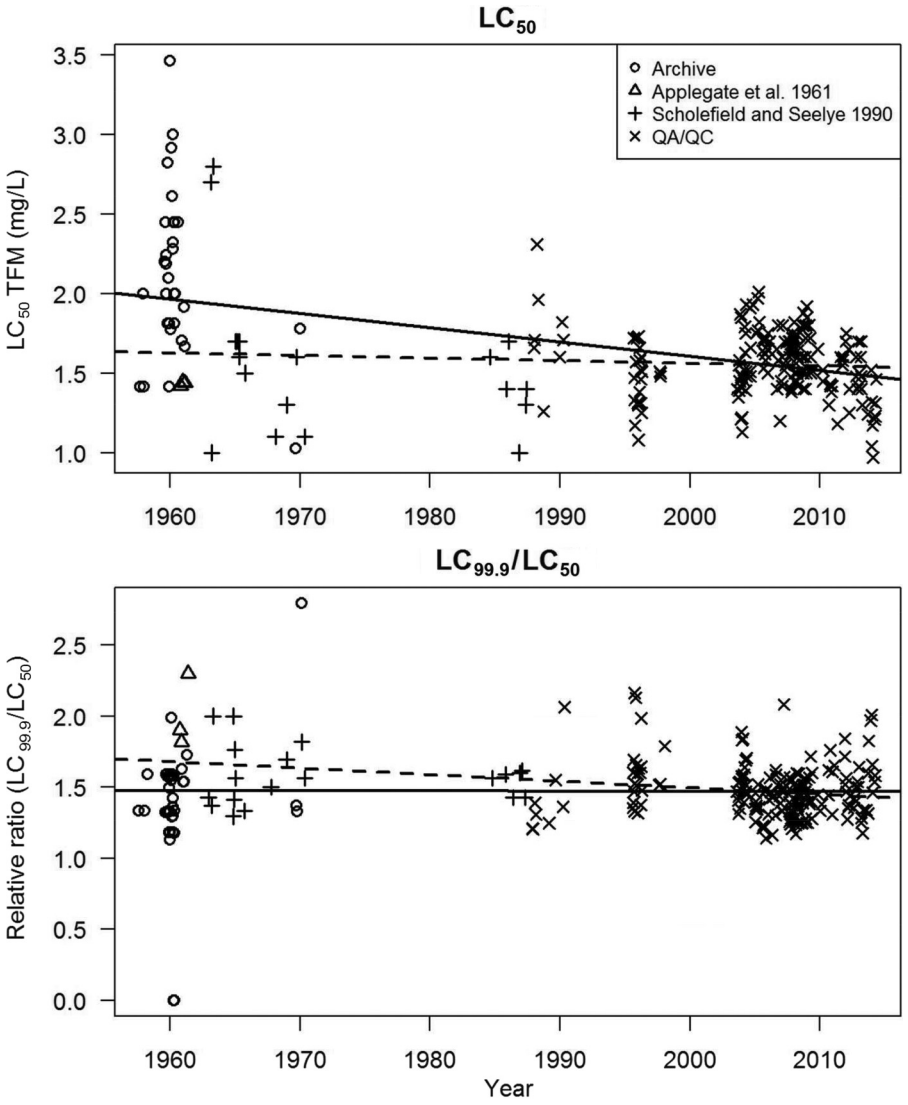
**Fig. A1.** Estimated reductions in juvenile parasitic sea lamprey recruitment to each of the Great Lakes, attributable to the effect of lampricide control, as indicated by the results of 1000 simulations of a sea lamprey operating model (Jones et al. 2009). Total heights of the bars are forecasted mean numbers of juveniles with no control; heights of the black segments are forecasted mean numbers of juveniles controlled at recent budget levels for each lake; the grey area represents the magnitude of reduction in recruitment due to control.




## Appendix B. Historical toxicity test analysis

We investigated the results of historical toxicity tests for evidence of physiological resistance to TFM in sea lamprey. The toxicity tests used in our analysis were conducted in the field in conjunction with stream treatments and in laboratories as part of ongoing research or chemical quality assurance tests. Information on the testing conditions (e.g., the pH, alkalinity, and temperature of the water) was not regularly recorded. To control for some of the potential variation in testing conditions known to influence toxicity tests (Bills et al. 2003), we focused our analysis on tests conducted at the US Geological Survey Hammond Bay Biological Station in Millersburg, Michigan. This laboratory uses Lake Huron water for its toxicity tests, which has relatively consistent pH and alkalinity (K. Slaght, personal communication). Toxicity tests at this facility are routinely run at 12 °C and occur between April and August. Seasonal differences in the lethal concentrations are not generally observed in the lab when conducting the toxicity tests because larvae are typically collected in the spring and held through the testing period (K. Slaght, personal communication). However, it is possible that these conditions were not always met in the earlier tests. Analysis was limited to

**Fig. B1.** Toxicity of 3-trifluoromethyl-4-nitrophenol (TFM) to larval sea lamprey exposed for 19–24 h in static toxicity tests in Lake Huron water conducted at the Hammond Bay Biological Station. Data were from archival records, historical reports (Applegate et al. 1961; Scholefield and Seelye 1990), and quality assurance tests (QA–QC) of TFM supplies (Adair and Sullivan 2014). Linear regression is shown fit to all data (solid line) and to all but the archival data (dashed line). A small random offset was added to sample from the same year to distinguish overlapping points. The estimated slope for $LC_{50}$ from all data was −0.009 (95% confidence interval: −0.011 to −0.007). Without the archival data, the $LC_{50}$ slope was −0.002 (95% confidence interval: −0.004 to 0.001). The estimated slope for the relative ratio from all the data was −0.00023 (95% confidence interval: −0.0020 to 0.0015). Without the archival data, the relative ratio slope was −0.0046 (95% confidence interval: −0.0067 to −0.0025). Six outliers ($LC_{50}$ or relative ratio > 3) from the archival records were removed; as these high values occurred early in the time series, their removal does not impact the sign of the slope.




static tests (between 19 and 24 h) because flow-through tests were not common prior to 1980.

We used historical toxicity test results from four sources (Table B1): (*i*) TFM concentrations and mortality counts from archival records; (*ii*) estimated lethal concentrations from Applegate et al. (1961); (*iii*) estimated lethal concentrations from Scholefield and Seelye (1990); and (*iv*) quality assurance tests of TFM supplies (Adair and Sullivan 2014) (Table 3). Applegate et al. (1961) did not report the year their toxicity tests were conducted, and we assumed all of their tests were conducted in 1961. The approach of Litchfield Wilcoxon (1949) was used to estimate the TFM concentrations at which 50% and 99.9% of the sample individuals died ($LC_{50}$ and $LC_{99.9}$, respectively).

Our analysis of historical toxicity data provided no evidence of resistance. The $LC_{50}$ of TFM did not increase over the 57-year time frame, nor did the relative ratio of $LC_{99.9}/LC_{50}$ increase (Fig. B1). An increase in $LC_{50}$ over time would have been the most obvious evidence that resistance has developed in sea lamprey exposed to TFM. If only a subset of the sea lamprey population were resistant to TFM, an increase in the relative ratio of $LC_{50}$ to $LC_{99.9}$ might be evident before an increase in the $LC_{50}$ was observed.

**Table B1.** Historical toxicity tests used to examine temporal trends in the lethal concentration of 3-trifluoromethyl-4-nitrophenol (TFM) required to kill 50% ($LC_{50}$) and 99.9% ($LC_{99.9}$) of sea lamprey larvae.

| Source | Year range | No. of $LC_{50}$ | No. of $LC_{99.9}$ |
|---|---|---|---|
| Archive | 1958–1970 | 38 | 38 |
| Applegate et al. 1961 | 1961–1961 | 3 | 3 |
| Scholefield and Seelye 1990 | 1963–1987 | 19 | 19 |
| Quality assurance and control tests (QA–QC) | 1988–2014 | 181 | 210 |

**Note:** Range of years and number of toxicity tests with estimates of the $LC_{50}$ and $LC_{99.9}$ are indicated.

There are several potential uncertainties with these toxicity data that limit the applicability of our findings as a test of physiological resistance to TFM. Our analyses relied on historical data that were collected for other purposes, not with the intent of specifically investigating the evolution of resistance. Sample sizes were low for some years, and key variables known to influence toxicity (e.g., pH, source stream of the larvae, temperature, timing of larval collection) were not always measured or controlled. Instruments used over time have also changed; for example, concentrations reported by Applegate et al. (1961) were measured using a Klett colorimeter, providing less precision than current methods (Adair and Sullivan 2014). Furthermore, the higher $LC_{50}$ values estimated from the archival data raise concerns (Fig. B1). While this pattern could indicate bias in the historical methods used to record TFM concentration, the cause is unknown. Thus, although we did not detect any overt signs that physiological resistance has developed, these results must be interpreted cautiously and further tests are needed.

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