

# Next-generation lampricides: a three-stage process to develop improved control tools for invasive sea lamprey

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**Abstract:** Successful integrated management of the invasive predatory sea lamprey (*Petromyzon marinus*) in the Laurentian Great Lakes of North America is owed largely to the long history of beneficial use of two lampricides: 3-trifluoromethyl-4-nitrophenol (TFM) and 2',5-dichloro-4'-nitrosalicylanilide (niclosamide). Ensuring continued successful sea lamprey control necessitates consideration of possible next-generation lampricides to supplement or replace current lampricides. This review identifies fifteen hallmarks of success for current lampricides to be used as design criteria in a search for next-generation lampricides. A three-stage research approach is outlined. Targeted research using omics, computer modelling, and high-throughput technology to define molecular mechanisms and high probability molecular targets for sea lamprey selective toxic action is crucial to prioritizing chemical candidates. Targeted delivery or identifying synergists to existing or new lampricides can provide increased efficiency and reduced environmental impact. Ultimate development of next-generation lampricides will rely on traditional toxicity testing methodologies to ensure safety and regulatory compliance.

**Résumé :** Le succès de la gestion intégrée dans les Grands Lacs laurentiens de l'Amérique du Nord de la lamproie (*Petromyzon marinus*), un prédateur envahissant, est en bonne partie dû à l'utilisation efficace et soutenue de deux lampricides, le 3-trifluorométhyl-4-nitrophénol (TFM) et le 2',5-dichloro-4'-nitrosalicylanilide (niclosamide). Le maintien de l'efficacité de la lutte contre la lamproie requiert l'examen d'éventuels lampricides de nouvelle génération comme compléments ou en remplacement des lampricides actuels. La présente synthèse cerne 15 aspects clés expliquant l'efficacité des lampricides actuels à utiliser comme critères de conception dans la quête de lampricides de nouvelle génération. Une approche de recherche à trois volets est décrite. Des travaux ciblés faisant appel aux sciences omiques, à la modélisation par ordinateur et à des techniques de haute capacité pour définir les mécanismes moléculaires et des cibles moléculaires présentant une forte probabilité d'action toxique sélective sur les lamproies sont d'importance clé pour la priorisation de substances chimiques candidates. La livraison ciblée et l'identification d'activateurs pour les lampricides actuels et de nouveaux lampricides peuvent rehausser l'efficacité et réduire l'impact environnemental. À terme, la mise au point de lampricides de nouvelle génération devra avoir recours à des méthodes traditionnelles d'essai de toxicité afin d'en assurer l'innocuité et la conformité à la réglementation. [Traduit par la Rédaction]

## 1. Introduction

Aquatic nuisance species are a continuing threat to ecosystems and the economic stability of regions centered around those ecosystems (<https://www.anstaskforce.gov>). One of the most significant invasive species control stories in North America is that of the invasive, parasitic sea lamprey (*Petromyzon marinus*; Fig. 1) in the Laurentian Great Lakes basin (Siefkes 2017). Sea lampreys invaded the Great Lakes in the late 1800s and early 1900s and parasitism of native fishes contributed to the devastation of the world class fishery including a catch reduction to 2% of historic levels. The first

highly successful sea lamprey control tools were de-facto and purpose-built barriers (e.g., concrete dams and electric weirs) that restricted adult sea lamprey access to upstream spawning and larval habitats, thereby limiting sea lamprey reproduction (Fig. 1) in certain tributaries (McLaughlin et al. 2007; Zielinski et al. 2019). For more than half a century, two lampricides, TFM (3-trifluoromethyl-4-nitrophenol) and niclosamide (2',5-dichloro-4'-nitrosalicylanilide), have been used to kill sea lamprey larvae below sea lamprey barriers and in undammed sea lamprey producing tributaries. Lampricide control has substantially reduced the Great Lakes sea lamprey

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**Fig. 1.** Sea lamprey (*Petromyzon marinus*) life cycle (inner rings). Available (\*) and potential future control methods are listed in the outer ring next to their life stage target. The sea lamprey is a jawless parasitic vertebrate native to the North Atlantic Ocean. Sea lamprey invaded inland freshwater ecosystems, including Lake Ontario, Finger Lakes, New York, and Lake Champlain via the Hudson River and New York State Canal System during the 1800s and Lakes Erie, Huron, Michigan, and Superior during the 1900s via the Welland Canal that bypasses Niagara Falls. Much of the life cycle occurs in tributaries except for the parasitic juvenile stage during which sea lampreys feed on host fish in open waters of invaded lakes. Parasitic juvenile sea lampreys attach to host fish with a toothed oral disc piercing the skin with a rasping tongue to ingest blood (Scott and Crossman 1973). The more temporally and spatially concentrated spawning adult, larval, and metamorphic stages are most susceptible to control. RNAi = RNA interference.



population to ~10% of historical levels and subsequently revitalized the aquatic ecosystem and economy, which supports 40 million people, including roughly 25% of the Canadian population (Siefkes 2017). Similarly, sea lamprey control benefits the fisheries of Lake Champlain and the New York State Finger Lakes (Marsden and Siefkes 2019). The binational (Canada and the United States) Great Lakes Fishery Commission (<http://www.glfc.org/>), operating through the 1954 Convention on Great Lakes Fisheries, was established to facilitate successful cross-border cooperation improve the Great Lakes fishery and oversee an integrated approach to sea lamprey control.

Unlike most of the agrochemical sector that has undergone continuous “greening” to meet societal and regulatory standards (Casida 2012), lampricides have maintained positive societal and regulatory approval because they are relatively species-specific, eliminated rapidly from the environment, and are effective. Furthermore, toxicity of lampricides to larval sea lampreys has remained relatively constant over time and ensured continued effective use (Dunlop et al. 2018). However, a growing emphasis on green chemistry (i.e., sustainable design) and integrated pest management necessitates a consideration of supplemental control options including next-generation lampricides. The green chemistry initiative demands consideration of potential deleterious effects from production through product use and environmental fate (Anastas and Warner 1998). The chemical control (e.g., small molecules

and biological control (e.g., RNA interference, pheromones) components of an integrated pest management strategy demand targeting multiple mechanisms of action for resistance prevention. As such, identification of alternative lampricidal mechanisms is a priority given that the continued use of one lampricidal mechanism imposes significant selective pressure for resistance development (Dunlop et al. 2018).

In this review, we explore the possibilities of “greening” next-generation lampricides (e.g., small molecules, RNA interference, pheromones) with enhanced characteristics compared to existing lampricides. Preferred next-generation lampricides could have desired characteristics in specificity, efficacy, economics, green chemistry, safety, or acceptability. An improved lampricide could garner greater regulatory and societal approval and ensure continued Sea Lamprey Control Program success. Our review builds on a recent review of sea lamprey physiology in relation to control in the Great Lakes (Siefkes 2017) and the population ecology underlying the contrasted status of Great Lakes invasive and European imperiled sea lampreys (Hansen 2016).

In line with the binational (Canada and the United States) Great Lakes Fishery Commission (<http://www.glfc.org/>) vision of an integrated approach to sea lamprey control on the Great Lakes, this paper aims to establish a strategy for identifying and developing next-generation lampricides. This contribution serves as a necessary update to the 1980 manuscript “Development of sea lamprey (*Petromyzon marinus*) larvicides” (Howell et al. 1980) and is

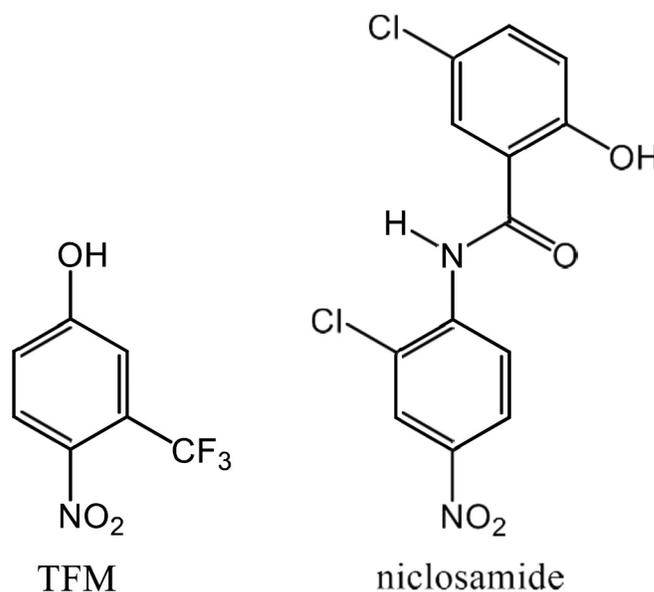
**Table 1.** Lampricide (3-trifluoromethyl-4-nitrophenol (TFM) and 2',5-dichloro-4'-nitrosalicylanilide (niclosamide)) formulations and their applications to invasive sea lamprey (*Petromyzon marinus*) control in the Laurentian Great Lakes.

Active ingredient	Formulation	Year introduced	Application
TFM, sodium salt	Liquid	1957	Sodium salt of TFM in liquid formulation used in lotic (stream) environments.
Niclosamide, ethanolamine salt	Wettable powder	1964	0.5%–2% niclosamide added to TFM applications in large, high-alkaline, and high-pH streams to reduce the amount of lampricide required while maintaining toxicity and selectivity.
Niclosamide, ethanolamine salt	Granular	1966	Coated granule targeted delivery formulation applied by a boat sprayer system that falls through the water column to release the active ingredient at the sediment surface. Applied in lentic areas (river mouths) and very large lotic systems (e.g., St. Marys River). The granular formulation is also used for sea lamprey larval abundance assessments.
TFM, sodium salt	Bar	1984	A solid, slow-release formulation used in small tributaries or side channels of lotic environments.
Niclosamide, ethanolamine salt	Emulsifiable concentrate	1998	0.5%–2% niclosamide added to TFM applications in large, high-alkaline, and high-pH streams to reduce the amount of lampricide required while maintaining toxicity and selectivity.
Niclosamide, ethanolamine salt	Bar	TBD	Under development by the United States Geological Survey as a possible TFM bar alternative with similar application.

especially relevant given 30 years of Sea Lamprey Control Program evolution and the advent of entirely new research technologies. Herein we (1) identify knowns and unknowns relevant to next-generation lampricide development and (2) recommend a conceptual research model for next-generation lampricide development. To achieve the first objective, we summarize historical information for the Sea Lamprey Control Program to establish necessary context; discuss lampricide mechanism of action; identify key next-generation lampricide characteristics including chemical design, delivery, and associated regulatory requirements; and outline research considerations including focus areas and technologies. To achieve our second objective, key findings from objective (1) are incorporated into a conceptual research model for next-generation lampricide development that centers on fifteen design criteria in six categories. The strategy presented is broadly applicable to 21st century pesticide development for any aquatic invasive species.

## 2. Fundamentals of sea lamprey control

The sea lamprey is an obligate stream spawner and has a larval stage that must reside in stream sediments for three or more years (Applegate 1950), which provides the basis of a control strategy centered on delivery of lampricides targeting the larvae in streams (Fig. 1; Applegate et al. 1961). In 1953, an intensive search began at what is now the United States Geological Survey's Hammond Bay Biological Station to find a chemical that was acutely toxic to larval sea lampreys at concentrations causing minimal damage to other aquatic organisms. Over 6000 compounds were systematically tested for toxicity to sea lamprey larvae, rainbow trout (*Oncorhynchus mykiss*), and bluegill sunfish (*Lepomis macrochirus*) (Applegate et al. 1957). The compound TFM, one of 10 tested had the desired differential toxic effects (Table 1; Fig. 2). The compound could be readily synthesized on a large-scale, had a desirable physical profile for field use, and proved more toxic to sea lamprey larvae than to other stream organisms, but toxicity varied with water chemistry (i.e., highest toxicity occurs under low hardness and low pH; Kanayama 1963). The screening effort also identified nitrosalicylanilides as potential lampricides with higher toxicity, but with less selectivity than TFM. Having the benefit of existing use as a molluscicide, niclosamide was chosen for development as a secondary lampricide (Table 1).

**Fig. 2.** Chemical structures of TFM (3-trifluoromethyl-4-nitrophenol) and niclosamide (2',5-dichloro-4'-nitrosalicylanilide), the two primary lampricides used to control invasive sea lampreys (*Petromyzon marinus*).

Lampricides and physical barriers are the two pillars of a successful Sea Lamprey Control Program. Additional physical and biological supplemental control strategies, such as pheromones, trapping, etc., are being explored but are not currently available for large-scale field application. Programmatic advances have dramatically improved lampricide application efficiency. Lampricide use has increased nonetheless, possibly indicating an increase in selective pressure over time. Historical toxicity data suggest that sea lampreys have not evolved heritable physiological resistance to TFM (Dunlop et al. 2018). However, lampricide resistance could arise through heritable life history or behavioral adaptation (Dunlop et al. 2018). The selective pressure imposed by lampricides is high and as the number of generations of sea lampreys exposed continues to grow, so too does the chance of

resistance evolution (Dunlop et al. 2018). Continued reliance on one lampricidal mechanism of action is inconsistent with integrated pest management chemical control standards, leaves the door open for resistance evolution, presents a clear risk to continued Sea Lamprey Control Program success, and provides impetus for next-generation lampricide development.

### 3. Lampricide mechanism of action

The lampricide TFM was first identified as an uncoupler of oxidative phosphorylation in isolated rat liver mitochondria (Niblett and Ballantyne 1976). Recent research demonstrated that TFM targets the inner mitochondrial membrane and reduces the proton motive force needed to drive proton flux through ATP synthase (Birceanu et al. 2011) causing marked reductions in anaerobic fuel stores such as glycogen and phosphocreatine during TFM exposure (Birceanu 2009; Birceanu et al. 2014; Clifford et al. 2012; Henry et al. 2015). Niclosamide impairs respiration and glucose uptake by cells and uncouples oxidative phosphorylation in non-fish models (e.g., human tumor cells; Park et al. 2011). The lampricides TFM (Birceanu et al. 2011) and niclosamide (Terada 1990) are commonly believed to be protonophores that reduce proton motive force by shuttling H<sup>+</sup> ions across the inner mitochondrial membrane. The phenolic structures of TFM and niclosamide (Fig. 2) provide a structural basis supporting their direct action as protonophores. Niclosamide is much more potent than TFM, causing death at lower concentrations without species specificity (Marking and Olson 1975; Dawson 2003). The physiological basis for the greater toxicity of niclosamide compared to TFM remains unresolved, but likely results in part from the greater lipophilicity of niclosamide. Specificity of TFM to lampreys is related to the relative inability of lampreys to detoxify TFM by the process of glucuronidation compared to most other fishes (Kane et al. 1994; Lech and Statham 1975).

Analysis of the recently assembled sea lamprey genome (Smith et al. 2013) indicated that sea lampreys express at least 2 isoforms of the gene encoding for uridine 5'-diphospho-glucuronyltransferase (Hlina et al. 2017), whereas at least 22 and 40 isoforms have been reported to date in humans (Bock 2016) and zebrafish (*Danio rerio*) (Wang et al. 2014), respectively. Phylogenetic analysis shows similar diversity in the uridine 5'-diphospho-glucuronyltransferase genes of other teleosts and amphibians (Huang and Wu 2010). Furthermore, a detailed enzyme kinetics analysis indicated that the maximal velocity and the affinity of uridine 5'-diphospho-glucuronyltransferase were substantially lower in sea lampreys compared to three nontarget fishes (Kane et al. 1994). A lesser contribution to TFM specificity may result from greater reductive metabolism in sensitive species including lampreys and lake sturgeon (*Acipenser fulvescens*) compared to other nontarget fishes (Bussy et al. 2015, 2018a, 2018b). Lack of niclosamide specificity could also be due to contributions of alternate metabolic pathways including sulfation and reduction (Dawson 2003) or fast niclosamide uptake rate driven by greater lipophilicity resulting in overwhelmed biotransformation.

Since inception of the Sea Lamprey Control Program, a better understanding has emerged about how TFM and niclosamide exert their lethal toxicity and this has translated to management level improvements. For example, treatments of high-volume streams are conducted using low percentage niclosamide and TFM in combination. The additive toxicity of the TFM–niclosamide mixture has similar selective toxicity to TFM alone and is of higher potency allowing for effective treatments with less chemical. However, we still have much to learn about how these compounds are absorbed, distributed, metabolized, and excreted by aquatic species as well as the chemical structural features responsible for differential action. Such knowledge of molecular determinants and resultant activity is necessary if we are to develop approaches to rapidly screen potential new sea lamprey toxicants for direct, synergistic or additive effects (see Section 6).

## 4. Next-generation lampricide

### 4.1. General characteristics

Next-generation lampricides will need to have ease of handling and application and high efficacy and safety under a wide range of environmental conditions to be appropriate for field use. Biodegradation, specificity for sea lampreys, and efficacy at low concentration are desirable characteristics of lampricides from an environmental perspective. The material should only persist as long as necessary to complete its intended biocidal activity. Extended persistence in the environment (days vs hours) increases the potential for effects on nontarget organisms. Human safety is critical, and it will be important to identify chemicals that have low mammalian toxicity and develop formulations that minimize risk of exposure to treatment personnel. Development and production costs are important considerations as the current Sea Lamprey Control Program expends nearly US\$6 million annually on lampricides.

### 4.2. Green pesticides

The lampricides TFM and niclosamide were developed during the 1950s, a time with little environmental regulation, little understanding of environmental impact, and considerable activity directed toward the synthesis of organic molecules for use in agricultural pest control (Norris et al. 2003). Both TFM and niclosamide have fewer environmental and human health risks than many pesticides made during the 1950s, a property that has been pivotal in their long history of regulatory approval. However, lampricide effects on nontarget fishes and invertebrates can occur under certain environmental conditions (Siefkes 2017). Natural pH fluctuation, high temperature and low dissolved oxygen levels in lampricide treated waters increase the possibility of nontarget effects. A responsibility to minimize environmental impact of lampricides drives our recommendation to explore green pesticides.

During the last two decades, the chemical industry has embraced the green movement. Green chemistry is defined “the utilization of a set of principles that eliminates or reduces the use or generation of hazardous substances in the design, manufacture, and application of chemical products” (Anastas and Warner 1998). Green chemistry starts with target molecule design and affects all aspects of chemical production from manufacture to use of the finished product. Even the most intensive alternative lampricide screening program is unlikely to identify a chemical that is completely environmentally benign and without risk. Thus, the expectation should be to identify chemicals with less environmental risk than TFM and niclosamide.

### 4.3. Delivery mechanisms

The delivery mechanism of next-generation lampricides should be carefully considered. Lampricide delivery at present is predominantly nonselective. During stream treatments, TFM or TFM–niclosamide mixture is dissolved in water and absorbed through the gill membranes of aquatic organisms including the targeted sea lamprey. However, the granular niclosamide formulation falls through the water column and once settled on the surface of the sediment, releases the niclosamide. As such, the granular niclosamide formulation is a targeted delivery system that minimizes exposure to nontarget organisms above the benthic zone within the application area. Refined targeted delivery including microencapsulation is used in the pharmaceutical and agricultural (Boehm et al. 2003) industries to enhance distribution of an agent to a particular tissue or organism. Microencapsulations that are sea lamprey selective could direct a lampricide dose to sea lampreys. This could increase efficacy of unstable or difficult to absorb payloads (e.g., RNA interference) or provide a basis for increased sea lamprey specificity thereby reducing effects to nontarget organisms. For example, detritus greater than 45 µm is a significant nutrient source for filter feeding larval Great Lakes sea lampreys (Sutton and Bowen 1994) in contrast to mollusks that live in similar habitat

and predominantly filter feed on smaller algae indicating a possible size exclusion threshold. Additionally, larval Great Lakes sea lampreys feed very slowly (Sutton and Bowen 1994), indicating a basis for enhanced selectivity via controlled release microparticle formulations. Targeted delivery of this type is being developed for control of invasive silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Hypophthalmichthys nobilis*), and common carp (*Cyprinus carpio*) in the Mississippi River watershed (Jon Amberg, USGS Upper Midwest Environmental Sciences Center, La Crosse, Wisconsin, USA, personal communication). Targeted delivery for existing and new lampricides is largely unexplored.

#### 4.4. Genetic control options

The prospect exists of producing an environmentally safe, sea lamprey-specific lampricide utilizing genetic technologies. Recent publications review possible applications of genetic technologies to sea lamprey control (McCauley et al. 2015; Siefkes 2017). A host of candidate genetic technologies are being rapidly developed (e.g., RNA interference, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), and the CRISPR-associated protein 9 (Cas9) system) and further researched for application to pest control problems. Perhaps the most prominent example of genetic control of a pest is gene-drive (where a particular gene or set of genes is favorably biased) via CRISPR-Cas9 for malaria control in mosquitoes (Gantz et al. 2015) or for mosquito vector control directly (Hammond et al. 2016). Gene-drives for malaria control showed great promise in laboratory tests, but direct vector control quickly faltered due to evolved resistance (Unckless et al. 2017). Feeding siRNA of the highly conserved elongation factor 1 $\alpha$ , calmodulin, and  $\alpha$ -actinin to sea lampreys effectively reduced transcript levels demonstrating RNA interference technology proof-of-concept for sea lamprey control (Heath et al. 2014). A critical need for either technology is a sequence of sea lamprey specificity in DNA for heritable gene drive (CRISPR-Cas9) or RNA (RNA interference). Considerable research is required if any of these techniques is to be seriously developed for sea lamprey control and substantial work will be required to determine if such techniques would obtain the social license for use (Thresher et al. 2019). Nevertheless, genetic technologies likely hold greater potential than small molecules for achieving selectivity between lamprey species — which is particularly challenging (see Section 5.3). While the emphasis of this review is on identifying targets for traditional small molecule pesticides, the approach could also help identify possible targets for genetic control.

#### 4.5. Regulatory requirements

Federal, state, and provincial regulatory requirements for next-generation lampricides will vary depending on the class of compound and its scope for use. Lampricide application is considered an aquatic non-food use by the United States Environmental Protection Agency (USEPA). Even natural-source compounds require some nontarget and ecological effects research to demonstrate safety. Pesticides with demonstrated reduced risk can undergo expedited USEPA registration (<https://www.epa.gov/pesticide-registration/conventional-reduced-risk-pesticide-program>). Registrants for reduced-risk products submit an executive summary, health and ecotoxicology data, a risk assessment, data for comparison to current pesticides used for the same purpose, and a description of proposed management practices to demonstrate (1) low human health risk, (2) low nontarget toxicity, (3) low potential for groundwater contamination, (4) low use concentrations, (5) reduced likelihood for the pest to evolve resistance, and (6) compatibility with an integrated pest management program. Beyond lampricide registration, the success of the Sea Lamprey Control Program relies greatly on collaborative relationships with federal, state, provincial, local, tribal or First Nation, and private entities throughout the Great Lakes basin. Permits, registrations, licenses, and access to land for program delivery are essential. The dedicated work of field program personnel,

researchers, and managers, as well as the high quality of the existing lampricide tools, contributes to a positive social license. Continual consideration of that social license is necessary from the earliest stages in the exploration for any potential next-generation lampricide.

## 5. Research considerations for next-generation lampricide discovery

### 5.1. Omics applications in next-generation lampricide research

Lampreys belong to one of two extant groups of jawless fishes, are distantly related to all other extant vertebrates (Smith et al. 2013; Docker et al. 2015) and possess many unique aspects of development and physiology. Omics technologies are a suite of tools for studying the roles, relationships, and actions of the molecules that make up the cells of an organism (<http://alttox.org/mapp/emerging-technologies/omics-bioinformatics-computational-biology/>). The publication of the sea lamprey genome (Smith et al. 2013) opened the door for identification of new lampricide targets. Freely available as a public resource, the 2.3 gigabase sea lamprey genome consists of 26 046 protein-coding genes on 84 pairs of chromosomes (Smith et al. 2013). Annotation of the genome (i.e., attaching biological information such as gene function to the sequences) included transcriptomic analysis, which measured gene expression in a wide variety of tissues and at several developmental points. Refined annotation of the sea lamprey genome is still needed and the draft Arctic lamprey (*Lethenteron camtschaticum*) genome, available through the Japanese lamprey Genome Project (<https://genomes.stowers.org/sealamprey>) provided additional genomic resources. A high-quality annotated reference genome assembly for the sea lamprey is nearly complete and is targeting a minimum metric contig N50 of 1 million bp (1 Mb), scaffold N50 of 10 Mb, 90% of the genome assembled into chromosome. The advent of next-generation sequencing genomic technologies, which can generate massive amounts of DNA or RNA sequence data from model and nonmodel organisms, is revolutionizing many biological disciplines. Genomics, proteomics, transcriptomics, and metabolomics are based on global, high-throughput analytical methods (e.g., multidimensional liquid chromatography coupled to tandem mass spectrometry to identify complex mixtures of proteins or small molecules). These tools are being used to revolutionize agricultural research (VanEmon 2016) and can be likewise applied to target identification for next-generation lampricide development.

### 5.2. Alternate mechanisms of action

Omics technologies can help identify lamprey-specific (and ideally, sea lamprey-specific) molecular targets to disrupt either basic physiological processes during the prolonged larval stage in tributary streams or one of the other highly specific, but temporally restricted stages of the lamprey life cycle (Fig. 1). Profound differences are known to exist between physiological systems and developmental stages of lamprey and other animals (Table 2), and examples are subsequently provided. Further directed research using omics technologies is ongoing and will provide additional invaluable information on exploitable differences as targets for lampricide development.

Analysis of the sea lamprey and Arctic lamprey genomes suggests that the immune and circulatory systems might provide unique targets for sea lamprey control. Adaptive immunity evolved independently in the jawless (lampreys and hagfishes) and jawed vertebrate lineages, and the two systems are fundamentally different (Boehm et al. 2012). Lampreys and hagfishes recognize antigens using variable lymphocyte receptors rather than the immunoglobulin-based B-cell and T-cell receptors found in all other vertebrates (Smith et al. 2013; Docker et al. 2015). Genes and proteins involved in the regulation of the variable lymphocyte receptor including an immunoreceptor tyrosine-based inhibitory motif containing immunoglobulin superfamily protein (Wu et al. 2016) could be lamprey-specific

**Table 2.** Physiological systems, life stages, and targets for potential selective lampricide action.

System or life stage	Target
Immune system	<ul style="list-style-type: none"> <li>Variable lymphocyte receptors</li> </ul>
Circulatory system	<ul style="list-style-type: none"> <li>Hemoglobin</li> <li>Clotting factors</li> </ul>
Embryogenesis stage	<ul style="list-style-type: none"> <li>Transition to exogenous feeding</li> <li>Programmed genome rearrangement</li> </ul>
Spawning adult stage	<ul style="list-style-type: none"> <li>Chemosensory sex pheromone receptors</li> <li>Migratory pheromone receptors</li> </ul>
Metamorphic stage	<ul style="list-style-type: none"> <li>Morphogenesis related to gills, mouth, intestine, etc.</li> <li>Lipogenesis</li> <li>Thyroid hormone receptors</li> <li>Hypothalamic–pituitary–gonadal axis (numerous)</li> </ul>
Reproductive system	<ul style="list-style-type: none"> <li>Gonadotropin-releasing hormone receptors</li> <li>Sex determination and sex differentiation</li> <li>Gamete production or viability</li> </ul>
Metabolic system	<ul style="list-style-type: none"> <li>Parasitic feeding associated traits</li> <li>Metabolic enzymes (specifically detoxification)</li> </ul>

Note: Discussion and abbreviations are included in the text.

targets. Similarly, hemoglobin evolved independently in jawless and jawed vertebrates (Docker et al. 2015) and could also provide a target-rich environment for selective lampricides. A specific loss of clotting-related genes in sea lampreys (Smith et al. 2013) indicates possibility for mechanisms that induce bleeding to be exploited in a taxon-specific manner.

High sea lamprey mortality occurs during the embryonic stage, particularly during the transition to exogenous feeding at approximately 30 days post-fertilization (Piavis 1961). Embryos may be especially vulnerable to novel lampricides. Much current research into embryological development uses lampreys as model organisms to study processes and gene regulatory networks that are conserved among vertebrates (Smith et al. 2010). Taxon-specific traits — most dramatically, a programmed genome rearrangement that occurs during lamprey embryogenesis (Smith et al. 2012) — should be further explored. Programmed genome rearrangement in sea lamprey embryogenesis results in the deletion of 20% of germline DNA in somatic cells and requires that the germline undergo meiotic recombination that increases potential misexpression of somatic cells similarly to oncogenesis (Smith et al. 2012). The possibly ancient nature of programmed genome rearrangement indicates that regulation of this process may be highly specific to extant jawless vertebrates although research has not yet defined specific regulatory points (Smith et al. 2012).

Considerable research effort has focused on semiochemicals, including pheromones, for sea lamprey control (Siefkes 2017). Genomic and transcriptomic analysis of sea lamprey chemosensory receptor genes (i.e., guanine nucleotide-binding protein-coupled receptors, GPCRs) shows some fundamental differences between lampreys and teleost fishes (Libants et al. 2009). Targeting these receptors may allow sea lamprey-specific disruption of chemosensory communication, which could be used, for example, to disrupt sea lamprey upstream migration and spawning. Semiochemicals could be used to bait traps and increase efficiency of

trapping for control (Li et al. 2007). The sea lamprey sex pheromone 3-keto-petromyzonol sulfate was the first vertebrate biopesticide approved for use by the USEPA and is being tested as a lure in traps and open streams (Johnson et al. 2013, 2015a, 2015b; Brant et al. 2016; Hume et al. 2015). A sea lamprey alarm has also been shown to manipulate adult sea lamprey behavior hastening their arrival at a trap site (Johnson et al. 2013, 2015a, 2015b; Brant et al. 2016; Hume et al. 2015). Combinations of pheromone antagonists have recently demonstrated proof of concept in disruption of pheromone-mediated reproductive behavior (Weiming Li, Michigan State University, personal communication).

Metamorphosis of parasitic sea lampreys involves radical morphological and physiological changes to virtually every organ system. Larval stage sea lampreys are largely sedentary, functionally blind, filter-feeders with flow-through ventilation. Parasitic juvenile stage sea lampreys utilize tidal ventilation that allows movement of water across the gills independent of the mouth, which has been adapted for host attachment and parasitic feeding (Fig. 1) (Manzon et al. 2015). Metamorphosis is preceded by lipogenesis (lipid levels increase from about 4% to 14% of the body weight) and includes complete remodeling of the intestine (during which time feeding is not possible), liver (including biliary atresia), and respiratory system, including a switch to an adult-type hemoglobin (Manzon et al. 2015).

Thyroid hormones are important for metamorphosis in lampreys, but the precise role of thyroid hormones in lamprey metamorphosis remains unknown (Manzon et al. 2015). Numerous regulatory points act collectively to control thyroid hormone action. The hypothalamic–pituitary axis regulates the synthesis and secretion of thyroid hormones from thyroid tissue (Manzon et al. 2015). Serum thyroid hormone distributor proteins transport thyroid hormone through the blood and regulate hormone availability (Manzon et al. 2015). Cytosolic deiodinases regulate thyroid hormone action via the conversion of (S)-thyroxine to the more biologically active (S)-triiodothyronine and the inactivation of both (S)-thyroxine and (S)-triiodothyronine (Manzon et al. 2015). The thyroid hormone nuclear receptors and the retinoid-X-receptors act as ligand-regulated transcription factors to modulate gene expression (Manzon et al. 2015).

Evidence suggests overlap in hypothalamic–pituitary–gonadal and hypothalamic–pituitary–thyroid axes in lampreys (Sower 2015). The hypothalamic–pituitary–gonadal axis is well-characterized as having multiple points of specificity in lamprey (Sower 2015). Further transcriptomic, proteomic, and metabolomic study will help characterize the dynamic shifts in genes, gene networks, proteins, and signaling molecules regulating metamorphosis, which could lead to effective and specific next-generation lampricides. Reproduction in all vertebrates is controlled by gonadotropin-releasing hormone (GnRH), a regulatory neurohormone. The hormone is produced in and released by the hypothalamus and acts at specific receptors in the pituitary gland to stimulate the synthesis and release of gonadotropin(s). Analogs of GnRH (both agonists and antagonists that, respectively, activate or block the GnRH receptor) are being used experimentally (Sower 2015). As simple decapeptides, GnRH molecules can be easily synthesized and different amino acids can be inserted at any position to produce analogs that can be more or less potent than the natural form. Given the uniqueness of the lamprey GnRHs (Sower 2015), it may be possible to design lamprey-specific analogs, either decapeptides or other chemicals that can agonize or antagonize neurohormone receptors (León-Olea et al. 2014). Neurohormones and GPCRs are widely exploited insecticidal and therapeutic targets (Hill et al. 2013) and provide proof-of-concept for sea lamprey GnRH GPCR target manipulation. A sea lamprey sterile male release tactic relied on direct injection of the nonselective chemosterilant Bisazir in a secure facility (Twohey et al. 2003; Siefkes 2017). Sterile males were deployed in Lake Superior during the late 1980s and in the St. Marys River from the late 1990s until 2011 (Twohey et al. 2003; Bravener and Twohey 2016). Sterile males

are currently being deployed experimentally in two low-density sea lamprey streams as a test of the technique's usefulness as a supplemental control tactic. Identification of a highly effective sea lamprey specific and environmentally benign sterilant affecting gamete production or viability for example might revitalize this program as a component of integrated sea lamprey management (Siefkes 2017). Although the role of the hypothalamic–pituitary system in lamprey reproduction has been well-characterized, other aspects of the genetic and molecular control of lamprey reproduction are less well-understood. These include the genetic basis of sex differentiation (the process by which the testes or ovaries develop from undifferentiated gonads) and sex determination (the genetic and (or) environmental “master switch” that activates the sex-specific developmental cascade that directs differentiation to proceed down one or the other pathway; Siegfried 2010; Docker et al. 2019). Evidence to date, however, suggests that the genes involved in sex differentiation are relatively conserved among vertebrates, including in lampreys (Spice et al. 2014), whereas those involved in sex determination are highly variable (McCauley et al. 2015; Johnson et al. 2017). Thus, manipulation of lamprey sex ratios toward males using genetic control methods (e.g., sex-ratio distorting gene drive systems; see above) likely holds greater potential for sea lamprey reproductive system specificity compared to small molecule design.

### 5.3. Lamprey species specificity

Current lampricides are not significantly selective for sea lampreys over native lampreys (Boogaard et al. 2003). Identification of next-generation lampricide targets with inherent selectivity for sea lampreys over native lampreys is particularly challenging. There are four native lampreys in the Great Lakes: the parasitic silver and chestnut lampreys (*Ichthyomyzon unicuspis* and *Ichthyomyzon castaneus*, respectively) and the nonparasitic northern brook lamprey (*Ichthyomyzon fossor*) and American brook lamprey (*Lethenteron appendix*) (Renaud et al. 2009). Selectivity may be achieved through disruption of the parasitic juvenile stage, particularly to the nonparasitic northern and American brook lampreys.

Omic technologies are being applied to the phenomenon of “paired” lamprey species (i.e., closely related parasitic–nonparasitic species pairs) to better understand the process by which sexual maturation is accelerated relative to metamorphosis in nonparasitic species, leading to elimination of the parasitic juvenile stage (McCauley et al. 2015). Although the parasitic sea lamprey does not have a known nonparasitic counterpart, other species pairs are being studied using next-generation sequencing technologies (Docker 2009). Genomic analysis of single nucleotide polymorphisms in a species pair in the Tagus River Basin of Portugal (European river lamprey (*Lampetra fluviatilis*) and European brook lamprey (*Lampetra planeri*)) revealed 166 fixed genetic differences between the parasitic and nonparasitic sister taxa (Mateus et al. 2013). The annotated sea lamprey genome was used as a reference to link 12 of these fixed differences to genes likely related to adaptation from anadromy to freshwater residency that, in this species pair, accompanied the transition from parasitism to nonparasitism (Mateus et al. 2013). The mechanisms and signaling molecules responsible for the “deletion” of the parasitic juvenile stage in nonparasitic lamprey likely hold specific control targets. Parasitic silver and nonparasitic northern brook lampreys have long been confined to fresh water and provide a model for genomic comparison. Similarly, the identification of metabolic enzymes unique to sea lampreys could provide a basis for differential metabolism of a purpose-built molecule. Designing a small molecule that has lamprey species specificity will likely be difficult and a genetic control tool (see Section 4.4) may be required to achieve.

**Table 3.** Categorized design criteria for next-generation lampricide development.

Category	Criterion
Specificity	<ul style="list-style-type: none"> <li>• High species selectivity*</li> <li>• Targeted delivery mechanism</li> </ul>
Efficacy	<ul style="list-style-type: none"> <li>• New lampricidal mechanism to minimize evolution of resistance*</li> <li>• Functional under a broad range of environmental conditions*</li> <li>• High potency</li> </ul>
Economics	<ul style="list-style-type: none"> <li>• Cost-effective discovery</li> <li>• Low production cost (synthesis and formulation)</li> <li>• Ease of delivery (field application)</li> </ul>
Environmental friendliness	<ul style="list-style-type: none"> <li>• No negative environmental effects from synthesis to use to environmental fate</li> <li>• Customized environmentally benign molecular structure</li> </ul>
Safety	<ul style="list-style-type: none"> <li>• No negative human health impacts</li> <li>• Low exposure risk to handlers and applicators</li> </ul>
Acceptability	<ul style="list-style-type: none"> <li>• Conform to regulatory standards</li> <li>• Favorable public perception (i.e., maintaining the social licence)</li> </ul>

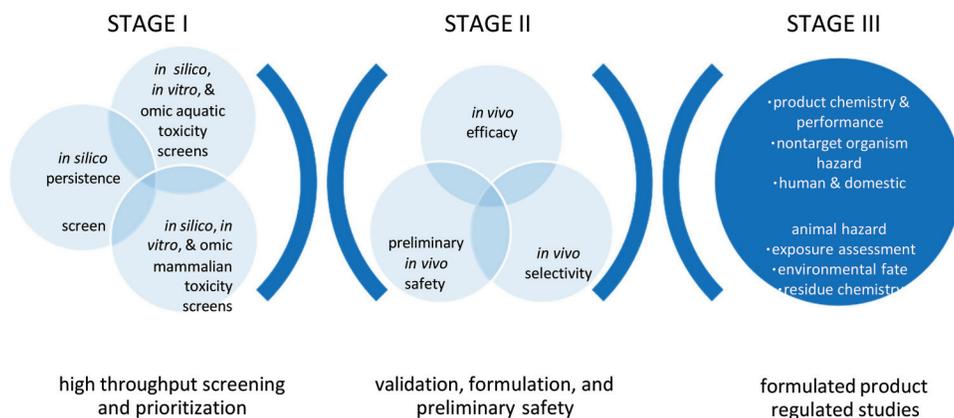
**Note:** Current lampricides perform moderately to very well at each criterion, but those marked with an asterisk (\*) are high priority for improvement in next-generation lampricide development.

## 6. A conceptual model for next-generation lampricide development

The 1950s lampricide screening endeavor (Applegate et al. 1957) identified relatively selective lampricides and subsequent research has answered many mechanistic questions. Modern technologies may enable informed discovery of next-generation lampricides selected to address high-priority deficiencies and desired improvements over current lampricides. We considered the success of and limitations of current lampricides and generated fifteen design criteria for next-generation lampricides (Table 3). A successful next-generation lampricide candidate might improve on only one or two of these criteria (e.g., new mechanism of action or production cost) to be high value, but consideration of all criteria during development will support a long use history. Recently, frameworks for comparison of green chemicals have been developed and will be useful in scoring promising candidates (DeVierno Kreuder et al. 2017).

Fundamental research described above will inform the structural, physicochemical, and biological characteristics of next-generation lampricides to evaluate candidate compounds against design criteria. We propose a three-stage conceptual model (Fig. 3) to guide the discovery and development of next-generation lampricides. This type of approach has been used in the agrochemical industry (Loso et al. 2017) but has not been applied to piscicide development. Stage I of our proposed model for next-generation lampricide development takes advantage of high-throughput technology including omics and in silico (computer) modeling to prioritize chemicals and classes for further study. Existing databases containing information about compound structure, chemistry, test condition, and biological effect could be used as a training

**Fig. 3.** Three-stage conceptual model for next-generation sea lamprey (*Petromyzon marinus*) lampricide discovery and development. Circles represent chemicals and classes meeting the success conditions for the identified studies. The circle overlaps represent the limited set of chemicals and classes expected to meet the combined requirements of the identified studies and progress to the next stage. Stage I consists of basic research using high throughput *in silico*, omics and *in vitro* screening tools to identify molecular targets and chemical classes. Chemicals and classes addressing a high significance design criterion (Table 3) are assigned higher priority. Stage II consists of *in vivo* assessment to validate the efficacy and selectivity of promising candidates. Formulation development occurs in Stage II as part of efficacy and selectivity studies. Preliminary (acute) mammalian toxicity studies for the active ingredient are also conducted in Stage II. Stage III consists of comprehensive regulated studies for a formulated product to support the pesticide registration.



set to build *in silico* screening tools (Buckler et al. 2005). The USEPA ECOTOX database (<https://cfpub.epa.gov/ecotox/>) is a substantial fish toxicity dataset that includes data from the fish toxicity project, the original lampricide screening data, and the University of Idaho piscicide screening project as well as numerous other sources.

The quality of *in silico* predictions depends on the training set and careful annotation and consideration of training set parameters is essential. Often overlooked aspects such as water quality can be critical to predictability as evidenced by the significant difference in TFM potency at varying pH and alkalinity. The best training sets include multiple chemicals within a class as evidenced by the reversal of selectivity or complete loss of potency resulting from minor alteration of the TFM chemical structure (Applegate et al. 1966). Understanding which parts of the molecule are responsible for the desired toxic action and which parts lead to undesirable effects or environmental persistence is essential to identifying an appropriate piscicide. The most basic *in silico* screen involves comparing chemicals against acute lethality. To that end, aquatic toxicity was predicted with moderate success by an *in silico* approach (Agatonovic-Kustrin et al. 2014). Once a screening tool is capable of reasonable predictions, chemicals untested for fish toxicity can be screened *in silico*.

Since the 1950s, millions of organic compounds have been synthesized or isolated from natural sources and new mechanisms of action have been discovered through intensive toxicological research. These new chemicals or new mechanisms of action represent unexplored avenues for next-generation lampricide development. More advanced *in silico* screens that incorporate mechanism of action (or “adverse outcome pathways”) are possible and have shown moderate success in pesticide mode of action predictions (Chiddarwar et al. 2017) and ecotoxicology (Madden et al. 2014). Targets of suspected sea lamprey specificity identified by omics — either for direct or synergistic action — can be screened against chemical libraries to identify chemical classes for further study. Preliminary considerations for compound synthesis and formulation should be made during Stage I. Finally, chemical classes identified as having aquatic toxicity would be run through *in silico* predictors for environmental persistence and mammalian toxicity (<http://www.epa.gov/nrmrl/std/qsar/qsar.html>) as the early identification of undesired nontarget effects is critical and would halt the further development of a compound (Hubert 2003). Results of *in silico* screens for efficacy, selectivity, persistence and safety

would be used to rank candidates for further study. Stage II of the proposed model for next-generation lampricide development utilizes high-throughput *in vitro* (cell) assays for refinement of efficacy and safety information in a biological system.

The purpose of this stage of the model is confirmation of *in silico* predictions or independent complementary results. Cytotoxicity assays using cells cultured from sea lampreys (efficacy), nontarget aquatic species (selectivity), and mammals (safety) can be incorporated into a high-throughput screening platform aimed at prioritization of lampricide candidates. The use of fish cytotoxicity assays is becoming increasingly popular to screen novel environmental pollutants and provide data that will inform environmental risk assessments (Fent 2001). However, correlation between *in vitro* cytotoxicity values and *in vivo* toxicity values is moderate and cytotoxicity observations are less sensitive than *in vivo* assay observations (Tanneberger et al. 2013). Thus more advanced target-based *in vitro* high-throughput screening is being applied in agrochemical research (Tietjen et al. 2005) and is of growing utility as an efficient screening tool. Functional profiling using model organisms such as yeast can identify novel mechanisms of toxicity (Gaytán et al. 2013) and be adapted to high-throughput screening. High-content *in vitro* screening platforms capable of measuring multiple endpoints simultaneously are also being developed (e.g., cardiomyocyte and hepatocyte toxicity) (Grimm et al. 2015). The Toxicity Testing in the 21st Century Initiative is widely embraced and is driving the development of new screening technologies as well as a fundamental understanding of molecular mechanisms (<https://ncats.nih.gov/tox21/>). This type of technology could greatly enhance the results of high-throughput *in vitro* screening for next-generation lampricides. Development of appropriate cell line(s) and exposure regimen to properly identify useful selective toxicants is crucial to success. Sea lamprey primary cell lines were initiated from gill, muscle, gut, brain, ovary, heart and kidney, but cell propagation beyond 8 months has not yet been achieved (Ma and Collodi 1999). Development of sea lamprey cell lines is currently being investigated in a collaborative effort between the United States Geological Survey and the US Fish and Wildlife Service.

Finally, supporting Stage II studies would define physicochemical properties of candidate compounds. Stage III of the proposed model for next-generation lampricide development consists of *in vivo* (live organism) platforms for ultimate efficacy and safety determinations, optimization, and regulatory approval. Live fish

bioassays remain a critical final stage in the development process of prioritized chemicals. The prospect of replacing standard fish toxicity assays for risk assessments with embryo toxicity screening tests amenable to high throughput has been considered extensively (Vergauwen et al. 2015). However, it is generally acknowledged that fish embryos are less sensitive to chemical exposure than bioassay-sized (0.1–5 g) or juvenile fish. Bioassay information at this stage must be of high reliability to inform effective and safe field application in varied environmental conditions. For this reason, standard fish toxicity assays with  $\geq 2$  g fish are required. Likewise, practical aspects of targeted delivery will be addressed using whole organism studies. Lastly, regulatory approval for any new pesticide will require a suite of standardized whole organism toxicity and environmental fate studies to establish safety.

## 7. Conclusions

A structured search for next-generation lampricides offers great opportunity to combine research initiatives and to advance basic science. The sea lamprey is just one of a host of aquatic invasive species of concern in the Great Lakes, and worldwide. Furthermore, the Aquatic Nuisance Species Task Force comprised of agency and regional representation from across the United States recommends readiness for quick action and responsible integrated pest management in the event of an aquatic nuisance species invasion (<https://www.anstaskforce.gov>). As of 2017, there are roughly two dozen USEPA registered active ingredients for aquatic use, of which two are lampricides, one is a pheromone, two are general piscicides, and the remainder are for control of other aquatic nuisance species including plants. A good return on investment would result from a tiered interdisciplinary research approach that uses high-throughput screening to rank potential study chemicals and mechanistic targets based on likelihood of identifying useful new lampricides. Further value would be added by simultaneously investigating selective toxicants for other aquatic invasive species.

Next-generation lampricide research and development should be conducted within the context of integrated pest management. New mechanisms of action are an absolute requirement if lampricide resistance develops. Rotation of lampricides with different mechanisms of action will decrease selection pressure and delay the onset of resistance. Furthermore, while TFM is relatively selective, enhanced selectivity toward target species should be a major consideration. Success of the original 1950s lampricide screen heavily relied on the contribution of chemical libraries by major chemical manufacturers in the private sector (Applegate 1957). Similarly, industry collaboration will be essential to the success of a next-generation lampricide screening program. The dataset generated by such an approach would be useful for identifying selective fish toxicants and the screening methods would be invaluable to the field of aquatic ecotoxicology. Information related to invasive sea lamprey control has considerable cross-over with that required for conservation of lampreys in their native ranges (Hansen et al. 2016). Developed omic, in silico, and in vitro platforms will be useful tools for identification and quantification of environmental impacts of chemical toxicants. Identified or refined adverse-outcome pathways will provide additional points of focus for ecotoxicology. The proposed approach of in silico prioritization followed by in vitro refinement and finally in vivo testing (Fig. 3) holds promise for rapid and cost-effective identification of invasive fish management chemicals.

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