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### **Relative Risk of Granular Bayluscide Applications for Fishes and Mussels of Conservation Concern in the Great Lakes Basin**

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## Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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## ABSTRACT

Bayluscide, a chemical lampricide, is used by government agencies in the Great Lakes basin to assess Sea Lamprey (*Petromyzon marinus*) as part of bi-national efforts to control the species. The use of granular Bayluscide has been highly successful in identifying and suppressing Sea Lamprey populations. However, the ecological risk of Bayluscide for fishes and mussels listed under Canada's *Species at Risk Act* and other species of conservation concern is not well understood. This document: 1) identifies potential pathways (direct, indirect) and mechanisms (physiological, non-physiological) by which the application of granular Bayluscide may influence fishes and mussels of conservation concern; 2) evaluates the relative ecological risk of applications based on four metrics (spatial distribution, application intensity, habitat associations that predispose species to exposure, toxicity); 3) identifies mitigation measures that may reduce potential impacts to non-target species; and, 4) identifies uncertainties needed to refine risk estimates. Spatial analyses indicated that between 2011 and 2017, Bayluscide applications occurred within the distribution of 21 fish and 15 mussel species of conservation concern including areas identified as critical habitat for 16 species (6 fishes, 10 mussels). For fishes, relative risk was greatest for native lampreys (Silver Lamprey [*Ichthyomyzon unicuspis*] and Northern Brook Lamprey [*Ichthyomyzon fossor*]) followed by Lake Sturgeon (*Acipenser fulvescens*) and Northern Madtom (*Noturus stigmosus*). Native lampreys exhibited high relative risk given habitat preferences and the toxicity of Bayluscide to those species. Northern Madtom ranked highly due to exposure (spatial and temporal patterns of application) and toxicity. Lake Sturgeon ranked highly due to exposure, though results were not directly comparable with other species due to assessment methods. For mussels, relative risk was greatest for Salamander Mussel (*Simpsonaias ambigua*), Threehorn Wartyback (*Obliquaria reflexa*), and Hickorynut (*Obovaria olivaria*). Salamander Mussel and Threehorn Wartyback ranked highly due to exposure and toxicity. A high risk ranking for Hickorynut occurred due to exposure and habitat preference. Mitigation measures to reduce the ecological consequences of Bayluscide applications are numerous and include modifying the frequency and timing of treatments, decreasing the size of application sites, and avoiding areas near critical habitat. However, the effect of potential mitigation measures should be rigorously tested to ensure that desired outcomes for species of conservation concern are realized while avoiding unintended consequences. Lastly, as reduced control effectiveness of Sea Lamprey would have undesirable effects on species of conservation concern susceptible to parasitism by Sea Lamprey (e.g., Lake Sturgeon), optimization measures may be warranted to account for such trade-offs.

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## INTRODUCTION

Sea Lamprey (*Petromyzon marinus*), a species native to the Atlantic Ocean, was first observed in Lake Ontario in 1888 and invaded the remaining Great Lakes between 1921 and 1937 following modifications to the Welland Canal (Smith and Tibbles 1980, Eshenroder 2014). Sea Lamprey caused widespread and significant mortality to fishes that support Indigenous, commercial, and recreational fisheries including Lake Trout (*Salvelinus namaycush*), Lake Whitefish (*Coregonus clupeaformis*), ciscoes (*Coregonus* spp.), and numerous other species. By the early 1960s, the commercial catch of Lake Trout in the upper Great Lakes fell from an average of 15 million pounds to 300 thousand pounds per year (Scott and Crossman 1973), a decline largely attributed to parasitism by Sea Lamprey. Early efforts to control Sea Lamprey led Canada and the United States to form the Great Lakes Fishery Commission (GLFC) in 1955 under the auspices of the *Great Lakes Fishery Convention Act*. Since then, the Commission has administered the integrated Sea Lamprey Control Program (SLCP) in cooperation with Fisheries and Oceans Canada (DFO), the U.S. Fish and Wildlife Service, and the U.S Army Corps of Engineers with the goal to reduce Sea Lamprey populations in the Great Lakes to levels that maintain or improve fisheries (Great Lakes Fishery Commission 1956).

Several tactics exist to control Sea Lamprey in natal streams ranging from purpose-built barriers and traps to the application of chemical lampricides. Evaluating the effect of control requires that Sea Lamprey populations be assessed on a recurring basis to determine population responses to control efforts including whether additional control sites should be considered. Assessment of Sea Lamprey populations to inform the control program involves sampling the depositional zone of nursery streams and other areas supporting Sea Lamprey production (e.g., connecting channels, some lake areas within the basin) to determine the incidence and abundance of larval Sea Lamprey. Standardized habitat classifications exist to guide assessment activities, which focus on habitat preferred by larval Sea Lamprey (Type I - composed primarily of silt substrates) or habitat used by larvae but not preferred (Type II - composed primarily of sand substrates) while avoiding habitat that is unsuitable due to larger substrates like gravel, cobble, and/or bedrock that deter burrowing (Type III).

The primary method to assess larval Sea Lamprey populations in wadeable streams involves backpack electrofishing (Slade et al. 2003). However, in some cases, deep (> 0.8 m) or turbid waters require alternate assessment methods to detect larvae such as the application of chemical lampricides (Weise and Rugen 1987). A chemical compound composed of 2', 5-dichloro-4'-nitrosalicylanilide or niclosamide ethanolamine salt (trade name Bayluscide; Dawson 2003) is regularly used for this purpose in granular formulation containing 3.2% active ingredient (hereafter referred to as gB). During application the granules are applied to plots  $\leq 500$  m<sup>2</sup> at a rate of 156 lbs/acre (175 kg/hectare) to approximate a Bayluscide concentration of 11 mg/L (9.3 mg/L active ingredient niclosamide [Adair and Sullivan 2004, Larval Assessment Task Force 2012]). When applied to the water's surface, the granules sink to the stream or lake bottom at an average rate of 0.07 m/s (United States Geological Survey [USGS] unpublished data), dissolve, and agitate larvae to swim from their burrows to the surface where they can be readily collected (Smith et al. 1974). Water temperature does not appear to have an effect on the release time of niclosamide from the granule (average of 3.64 mins). However, fewer Sea Lamprey larvae emerge in temperatures less than 12°C within 1 hour of application (Boogaard et al. 2016a). In some cases, application of gB may be used as a control tactic in deepwater habitats (e.g., St. Mary's River) where conventional applications of the lampricide TFM (3-trifluoromethyl-4-nitrophenol; Hubert 2003) would be ineffective or overly costly.

The use of gB in the Great Lakes basin has been highly successful in detecting and suppressing larval Sea Lamprey populations and remains an important component of the

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bi-national control program. However, given the known toxicity of Bayluscide to non-target species (Dawson 2003, Boogaard et al. 2016b, Newton et al. 2017), concern exists about the potential for direct and indirect effects on fish and mussel species of conservation concern within the Canadian waters of the Great Lakes basin. Although previous studies have evaluated the toxicity of Bayluscide to a range of non-target organisms (Marking and Hogan 1967, Bills and Marking 1976, Gilderhus 1979, Scholefield and Seelye 1992), impacts to species of conservation concern in Canada have not been widely addressed based on the suite of factors that may influence species responses in the wild (e.g., habitat associations that predispose species to exposure; food-web effects).

In 2011 and 2012, concerns were raised by managers within DFO's Species at Risk program regarding the application of gB in several areas of southwestern Ontario inhabited by fish and mussel species listed under Canada's *Species at Risk Act* (SARA) including the Detroit, St. Clair, Sydenham, and Thames rivers and Lake St. Clair. Each system contains a unique assemblage of SARA-listed species (e.g., Detroit River: Northern Madtom [*Noturus stigmosus*], SARA Endangered; Channel Darter [*Percina copelandi*] [Lake Erie Designatable Unit], SARA Endangered; Spotted Sucker [*Minytrema melanops*], SARA Special Concern). As a result, staff from DFO Species at Risk Program, DFO's Sea Lamprey Control Centre (SLCC), and the Great Lakes Laboratory for Fisheries and Aquatic Sciences (GLLFAS) identified the need to better understand the ecological risk of gB applications for species assessed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) as Endangered, Threatened, or Special Concern as well as those listed as Endangered, Threatened, or Special Concern under SARA.

Scientific advice about the ecological risk of gB applications on SARA-listed species is needed for several reasons. First, DFO's Species at Risk Program is responsible for identifying threats to SARA-listed species or species under consideration for listing within the federal Species at Risk recovery planning framework. Threat identification and evaluation requires understanding how the risk of gB applications (if any) compares to other pertinent threats. Threat identification and evaluation (DFO 2014b) is also used to guide the development of federal recovery strategies, noting research and recovery actions needed to mitigate key threats. Second, Section 73(3)(c) of SARA stipulates that the competent Minister may grant SARA permits for works/undertakings/activities (w/u/a) "...only if the competent Minister is of the opinion that the activity will not jeopardize the survival or recovery of listed species", raising into question the ecological effects of gB from a regulatory perspective. To support the interpretation of Section 73(3)(c) across a range of w/u/a, DFO Science provides 'allowable harm' advice to the Species at Risk Program defined as the maximum harm that can be applied to a listed species without jeopardizing its survival or recovery (e.g., see van der Lee et al. 2020). Allowable harm advice is usually provided as the mortality rate that would not lead to a declining population trajectory. However, all types of harm (changes in growth, habitat effects leading to changes in reproductive output) are relevant if they influence the productivity of the species. In most cases, the interpretation by DFO Science has been that only growing populations have scope for harm as harm to declining populations would jeopardize recovery and thus is inconsistent with Section 73(3)(c) (e.g., van der Lee et al. 2020).

## **BAYLUSCIDE PATHWAYS OF EFFECTS**

There are multiple ways that toxicants in the environment can affect aquatic organisms. Many studies have evaluated how toxicant exposure can lead to physiological changes that influence survival and growth. Several toxicants are known to cause organ and cell damage in fishes and mussels and may affect the central nervous system, disrupting physiological processes such as respiration (Sprague 1971, Widdows and Page 1993) and decreasing survival (McKim et al. 1974). Indirectly, toxicants can decrease the resilience of fishes and mussels to other stressors

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(Sprague 1971, Holmstrup et al. 2010) such as temperature (McKim et al. 1974, Brungs et al. 1978) and disease (Austin 1999). Contaminants can directly reduce growth rates through the disruption of metabolic processes or indirectly through changes in prey availability, though the effect of toxicants on fish growth is variable across contaminants where exposure can result in decreased, increased, or no change in growth (Sprague 1971). Organism movement, both large (e.g., migration) and small scale (e.g., predator avoidance), may be disrupted following toxicant exposure in both fishes and mussels (Sprague 1971, McKim et al. 1974, Austin 1999, Hazelton et al. 2014) by altering active oxygen uptake (Sprague 1971) and/or oxygen utilization by cells (Brungs et al. 1978). Reduced oxygen uptake can decrease swimming performance, reduce the efficiency of food capture, or lower the ability to evade predators (Sprague 1971), which may indirectly influence growth and survival. Despite general evidence of declines in movement following toxicant exposure, some toxicants, such as Dichlorodiphenyltrichloroethane (DDT), can lead to hyperactive locomotive behaviour in fishes (Brungs et al. 1978) and chronic fluoxetine exposure has led to increased movement in freshwater mussels (Hazelton et al. 2014). Conversely, several toxicants have been found to prevent an avoidance response in fishes (Sprague 1971), which may result in decreased survival when predators are present. Changes to reproductive output due to life history impacts such as the delay of sexual maturity (Sprague 1971) or changes in behaviour such as increased duration of courtship (Jones and Reynolds 1997) have also been linked to exposure to various toxicants. The likelihood of successful reproduction can also be reduced as egg production by fishes may decline or cease in the presence of certain toxicants (Sprague 1971, Jones and Reynolds 1997). In the case of freshwater mussels, certain chemicals such as selective serotonin reuptake inhibitors have the potential to disrupt reproduction by influencing the timing of reproductive behaviours (Bringolf et al. 2010). Although the organism responses described here have been documented under various ecological conditions, the types of responses including the severity of changes to growth, survival, or reproduction will depend on numerous contaminant-specific, ecosystem-specific, and species-specific factors.

Based on the general effects of toxicant exposure identified in the literature, Bayluscide applications may have the potential to influence fish and mussel species of conservation concern through various pathways, both directly and indirectly (Figure 1). Direct effects, defined as those acting principally on focal species, may include changes to vital rates such as mortality, growth, reproductive potential, and movement/migration, which can influence production including population trajectory, abundance, and persistence of the species in question. Indirect effects, defined as those acting on food-web components, may impact the vital rates of prey, predators, and competitors leading to Bayluscide-induced responses for species of conservation concern due to linked food-web effects. A unique case of indirect effects may exist for freshwater mussels where changes to the vital rates of host organisms, which are required to complete the obligate parasitic life stage, may alter reproductive potential.

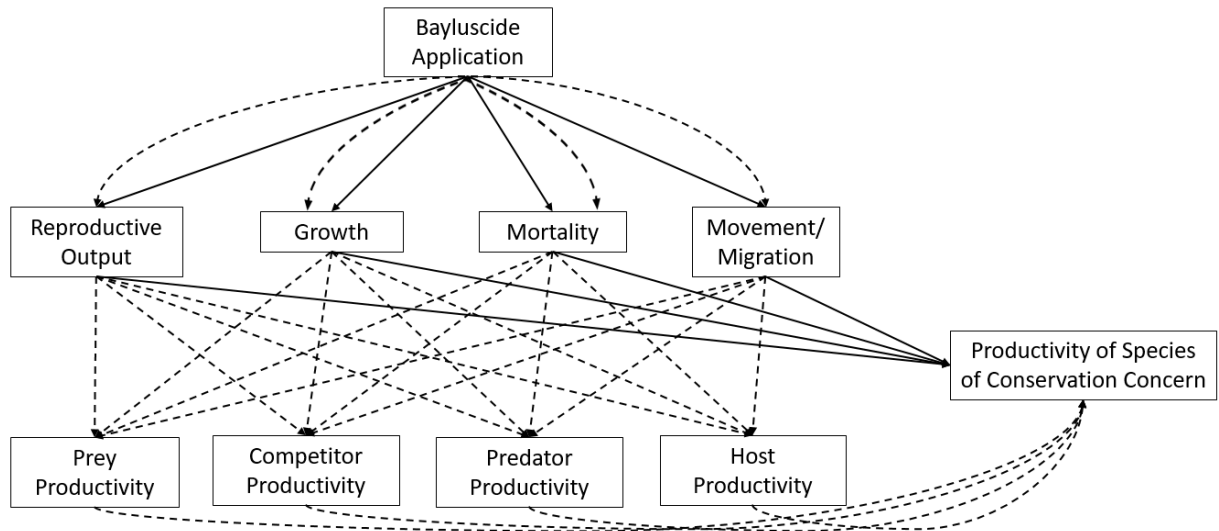


Figure 1. Bayluscide Pathways of Effects for species of conservation concern, including direct (solid lines) and indirect (dashed lines) pathways. Both direct and indirect pathways may influence species of conservation concern through physiological (e.g., toxicity) and non-physiological (e.g., avoidance) mechanisms.

Direct and indirect pathways involving Bayluscide may influence aquatic organisms through physiological and non-physiological mechanisms. Physiological mechanisms involve Bayluscide-induced shifts in mortality, growth, reproductive output, or movement stemming from the physiological effect of the compound on cellular or individual processes. For example, mortality or growth may be altered due to the uptake of the compound and changes in metabolism or organ function required for growth or survival may occur. In other cases, reproductive output may be affected if toxicity of the compound leads to reduced egg development or viability. The manner in which an organism responds to a chemical will depend on its mode of action. The mode of action of niclosamide, the active ingredient of Bayluscide, is believed to be through the impairment of oxidative phosphorylation or stimulation of ATPase activity (Vinaud and Lino Junior 2017). For example, in a freshwater snail, exposure to Bayluscide led to a decrease in the number of mitochondria as well as a number of other intracellular changes, causing cellular damage (Xiong et al. 2016). Due to its toxic effects on cellular respiration, selective responses between Sea Lamprey, mussels, and other aquatic organisms may be limited, though Dawson (2003) identified species-specific differences in toxicity for numerous aquatic organisms. However, additional research is required to better understand the mode of action of niclosamide and resulting species responses (Zhang et al. 2015, Smyth and Drake 2021).

In addition to physiological mechanisms, non-physiological mechanisms can also cause Bayluscide-induced changes in behaviour or vital rates. If organisms detect and avoid Bayluscide in the environment (see Boogaard et al. 2008b, Boogaard et al. 2016b), disrupted behaviour or movement from the application site may result in vital rate shifts as a consequence of disturbance. For example, avoidance may result in Bayluscide-induced mortality if avoidance increases predation risk or leads to occupancy within sub-optimal ecological conditions. Avoidance may also lead to shifts in reproductive output if spawning activity is foregone or takes place within sub-optimal habitat. In mussels, avoidance behaviour is largely confined to valve closure in response to a toxicant. Although this behaviour can mitigate the short-term effects of toxicity, it comes at the cost of decreased feeding and respiration.



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Importantly, direct and indirect pathways do not necessarily result in negative effects. The pathways outlined in Figure 1 illustrate potential routes by which Bayluscide applications may influence each ecosystem component but do not imply directionality or intensity of pathways and species responses. It is possible that individual pathways may have unexpected beneficial effects on species of conservation concern. For example, Bayluscide-induced mortality on the predator of a SARA-listed species may relax predation pressure and increase survival but would only lead to a net benefit if predation superseded all other direct and indirect pathways acting on the focal species. A distinct case of beneficial effects involves the increased ability to detect and suppress Sea Lamprey populations as a result of Bayluscide applications, which benefits large-bodied species of conservation concern (e.g., Lake Sturgeon [*Acipenser fulvescens*]) susceptible to Sea Lamprey-induced mortality and non-lethal effects (e.g., wounding). Therefore, the overall effect of Bayluscide applications on species of conservation concern will be the aggregate effect of each direct and indirect pathway that considers all underlying physiological and non-physiological mechanisms.

Understanding the response of fishes and mussels to gB applications through direct and indirect pathways is extremely challenging and requires knowledge of Bayluscide-specific, ecosystem-specific, and species-specific factors. These include:

1. the likelihood of species exposure to a given concentration of Bayluscide, which is a function of:
  - a. the spatial and temporal distribution of gB applications,
  - b. the spatial and temporal distribution of species whether focal, prey, predator, competitor, or host,
  - c. characteristics of the application (e.g., concentration of Bayluscide in the aquatic environment per unit time and space as dictated by application rate, fluvial conditions, and other environmental factors),
  - d. characteristics of the aquatic ecosystem that allows Bayluscide to interact with a given species (e.g., habitat associations that predispose species to exposure; water temperature);
2. the response of organism(s) to Bayluscide concentrations in the environment, whether physiological or non-physiological, including:
  - a. the likelihood of avoidance and
  - b. the dose-response relationship for a given concentration plus the magnitude of individual vital rate shifts; and,
3. how acute or chronic responses, individually and collectively, influence the productivity of species.

Given the above factors, it is expected that different species will have different responses to gB due to the number of pathways involved and the overall effect of contaminant-specific, site-specific, and species-specific factors.

Due to low population densities and the difficulty of measuring life-history parameters with non-lethal methods, significant data gaps surround the ecology of many species of conservation concern. These include basic knowledge of species ecology (e.g., age structure; age-specific vital rates) and uncertainty in how food webs promote species persistence (incomplete knowledge of prey resources, uncertainty about the strength and consequences of competitor or predator effects including how shifts in food web components would shift the productivity of focal species). These factors have bearing on understanding how non-physiological mechanisms

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may influence vital rates (e.g., the consequence to survival or growth of shifts to sub-optimal habitat; ability of species to re-colonize after disturbance). Knowledge gaps also exist about many Bayluscide-specific factors underlying the pathways in Figure 1, such as spatial and temporal variation of Bayluscide concentration in the aquatic environment and dose-response relationships for species of conservation concern and their relevant food-web components. Therefore, a comprehensive, mechanistic understanding of each direct and indirect Bayluscide pathway is unlikely to occur even for the best-studied species. The Bayluscide Pathways of Effects (Figure 1) were developed to illustrate the difficulty of predicting and generalizing species responses and to emphasize that indirect effects and those not physiological in nature are likely relevant for understanding the consequence of Bayluscide exposure for species of conservation concern. This document focuses on a subset of relevant pathways presented in Figure 1. However, the resulting classification framework should be used to identify effects requiring future research and to establish common terminology when describing the effects of Bayluscide on fish and mussel species of conservation concern.

## **SCOPE AND OBJECTIVES**

This research document identifies fishes and mussels of conservation concern, which includes fishes and mussels assessed by COSEWIC as Endangered, Threatened, or Special Concern, as well as those listed under SARA as Endangered, Threatened, or Special Concern (as of May 2019) in the Canadian waters of the Great Lakes basin, that would have a reasonable chance of being exposed to gB based on patterns of past applications. The primary objective was to evaluate the relative ecological risk of gB applications on fishes and mussels of conservation concern, focusing on the direct physiological pathway based on four lines of evidence: the 1) distribution and 2) intensity of gB applications in relation to the distribution of fishes and mussels of conservation concern; 3) habitat associations that predispose fish and mussel species to direct exposure within application sites; and, 4) the toxicity of gB to fish and mussel species including surrogate species where appropriate. Spatial analyses were used to summarize the proximity of gB applications to each species including whether gB has been applied within areas containing critical habitat for species listed under SARA.

The methods and results of the relative risk assessment are presented first followed by species accounts. Species accounts summarize the distribution and habitat of species that could potentially influence species responses. Species accounts also provide an overview of allowable harm as estimated by DFO's Science Sector in Recovery Potential Assessments, and identify known or potential impacts of gB to inform Pathways of Effects based on a search of current literature. Lastly, each species account reviews the species-specific results of the relative risk assessment. The final two sections of this document identify mitigation measures and describe overall uncertainties. Mitigation measures have been identified that may reduce the scope for direct and indirect effects during applications, should risks be deemed non-negligible for fishes and mussels of conservation concern.

This document is intentionally broad in scope and geographic coverage with analyses focusing on the relative risk of exposure and direct mortality across the Canadian waters of the Great Lakes basin. An accompanying research document (Smyth and Drake 2021) provides quantitative estimates of the potential for mortality stemming from the direct physiological pathway for a subset of species within four focal rivers in southwestern Ontario. Together, these documents can be used to understand relative risk among species in Canadian waters (this document) and to gauge the potential for Bayluscide-induced mortality for a single focal tributary undergoing an application cycle, including the effects of changes to application variables (site area, site number, and frequency of applications; Smyth and Drake 2021).

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## RELATIVE RISK ASSESSMENT

A risk assessment was developed to evaluate the relative ecological risk of direct mortality to fishes and mussels of conservation concern stemming from physiological effects. The risk assessment was based on four metrics: 1) the proportion of the species' range susceptible to gB applications; 2) the intensity of applications within the species' range; 3) habitat associations that predispose species to exposure (i.e., preferential occupancy within Type I or Type II habitat); and, 4) standardized toxicity of gB to fishes and mussels based on focal or surrogate species. Individual metrics, which were derived between 0 and 1 (described below), were selected as the most reasonable proxy variables to describe relative differences in the likelihood of exposure (distribution, intensity, habitat associations) and toxicity among species in the Great Lakes basin. The relative risk of direct mortality,  $RR_M$ , was calculated as  $RR_M = R \times I \times H \times T$  where  $R$  represents the species' range variable,  $I$  represents intensity,  $H$  represents habitat associations, and  $T$  represents toxicity. Multiplication was used to estimate  $RR_M$  due to the conditional nature of each contributing process. Untransformed values of  $RR_M$  range from 0 (low relative risk) to 1 (high relative risk) with values close to 1 reflecting a species with high distributional overlap, high relative application intensity, high preference for Type I or Type II habitat, and high relative toxicity to gB. Because of uncertainty in how these factors influence direct mortality, equal weighting among variables  $R$ ,  $I$ ,  $H$ , and  $T$  was assumed to be the most reasonable estimation method. However, alternative weightings are possible. Individual variables ( $R$ ,  $I$ ,  $H$ ,  $T$ ) provide useful stand-alone components that may be used to evaluate additional factors about the relative risk of gB applications. For example, omitting  $R$  and  $I$  from the relative risk equation would provide the relative risk of direct mortality if gB applications are expected to occur randomly, relative to species at risk (SAR) distributions.

Relative risk was calculated for all species of conservation concern. The derivation of each assessment metric, including geospatial analyses to support variables  $R$  and  $I$ , is described below.

## GEOSPATIAL ANALYSES

To identify the species most likely to be impacted by gB application within the relative risk assessment, several spatial analyses were performed. The primary goal of these analyses was to determine the proximity of gB applications to fish and mussel species of conservation concern, but secondarily to determine the degree of spatial co-occurrence between larval Sea Lamprey populations and focal species as well as the extent to which gB applications have occurred in areas defined as critical habitat under SARA. Given the low detection rates and small number of records available for many species at risk, mapping of gB applications within critical habitat was one way of identifying potential impacts to species. Spatial analyses were used to evaluate the basis for exposure based on spatial patterns of past gB applications with the assumption that future applications may exhibit similar spatial patterns and thus similar likelihood of spatial exposure. The primary objective was performed by identifying the proportion of the fish or mussel species distribution that contained or was in close proximity to gB applications, thereby determining the fraction of the species' range potentially affected when applications occur. However, we also identified the proportion of gB application sites in proximity to a fish or mussel species, thereby determining the fraction of gB sites with potential conservation concern.

To perform the analyses, distribution records of focal fish species were mapped using data from DFO's Biodiversity Database (DFO unpublished data) which encompasses > 600,000 field collections made by DFO Science as part of SARA-related research activities as well as data received from SARA permit holders plus numerous historical species inventory records (e.g., Canadian Museum of Nature, Royal Ontario Museum). Sea Lamprey and native lampreys

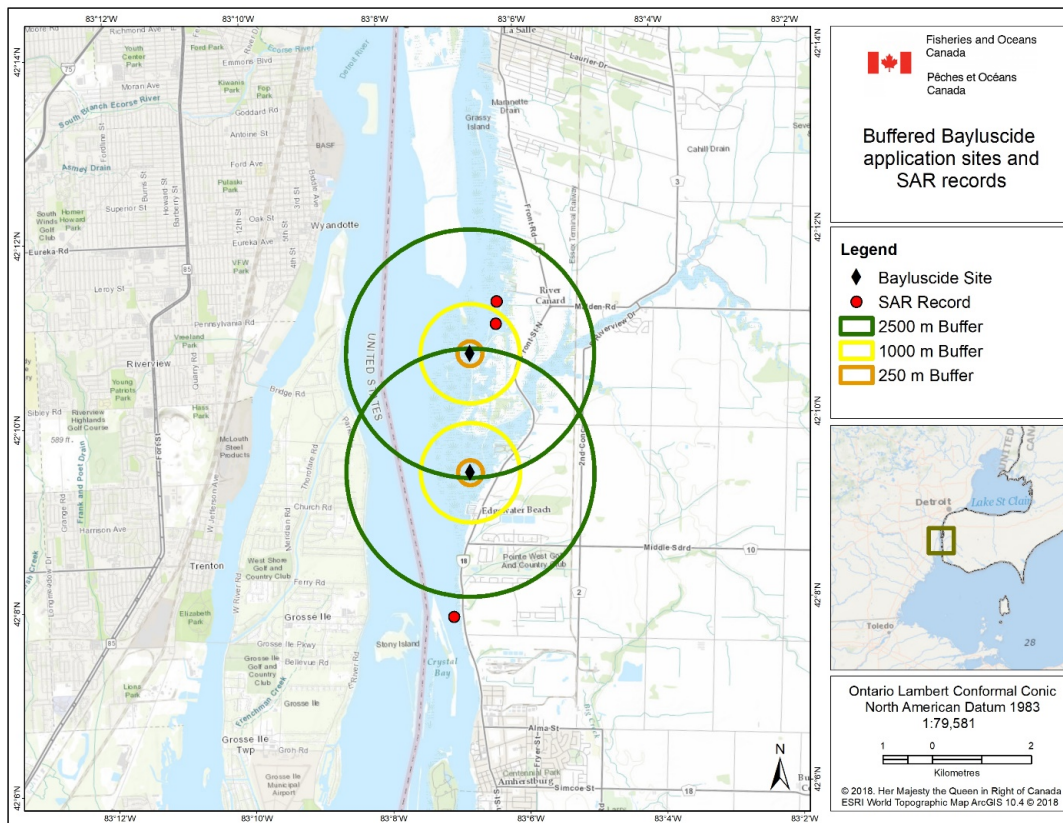
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collected by DFO's SLCC based in Sault Ste. Marie, Ontario were also included in spatial analyses. Maps of species co-occurrence with Sea Lamprey were only included in this document when both Sea Lamprey and a species at risk were found to have overlapping ranges. Due to the difficulty of differentiating native lamprey species as larvae, the majority of native lamprey records within the databases were classified as *Ichthyomyzon* sp., signifying either Silver Lamprey (*I. unicuspis*; SARA Special Concern), Northern Brook Lamprey (*I. fessor*; SARA Special Concern), or Chestnut Lamprey (*I. castaneus*; assessed by COSEWIC as data deficient). In some cases, definitive species-level identifications were made, usually signifying the collection of adults. To incorporate species-specific and unspecific native lamprey occurrence records, two approaches were incorporated. The first involved evaluating Northern Brook Lamprey and Silver Lamprey using only records definitively identified to species level. A second evaluation for each lamprey species was conducted by combining the species-specific records (definitive Silver Lamprey or definitive Northern Brook Lamprey) with all remaining *Ichthyomyzon* sp. records. These approaches likely underestimated (definitive species-level records) and overestimated (species-level records + unidentified records) the range of each species. Attempts were made to determine species membership for unidentified records based on stream types (small streams or those above barriers, likely Northern Brook Lamprey; large streams and below barriers, likely Silver Lamprey), but definitive species-level collection records indicated that both species have been documented in both habitat types so the species and species + unidentified approach was retained.

Records from DFO Science were used to map mussel species distributions including records from quadrat sampling and timed search sampling (see Metcalfe-Smith et al. [2000] and Metcalfe-Smith et al. [2007] for details). For mussels, records of live and fresh specimens were included but records from extirpated populations were omitted. For both fishes and mussels, distribution records were included from 1998 onwards. Species occurrence records from within Walpole Island First Nation lands were omitted from map figures in this document due to the conditions of a data sharing agreement. The distribution of Sea Lamprey ammocoetes (being the target of gB applications) was also mapped using data collected from 2011 to 2017 by DFO's SLCC. The spatial distribution of gB application locations in the Canadian tributaries of the Great Lakes basin, also covering the period from 2011 to 2017, was identified based on data from DFO's SLCC. Only granular applications (i.e., those in the absence of TFM), whether for assessment or treatment/control of Sea Lamprey, were considered in the analyses.

The distribution of fishes and mussels was mapped as individual collection records as well as by identifying Ontario Ministry of Natural Resources and Forestry's Aquatic Landscape Inventory System (ALIS; Stanfield and Kuyvenhoven 2005) segments that contained or were located within 250 m of a distribution record. ALIS segments, which are geographic polylines reflecting the ecological similarity of stream and river segments in Ontario, were buffered by 250 m as one way of ensuring that distributional overlap with gB was not biased by sampling factors. This approach helped account for imperfect detection of fish and mussel species and the resulting incomplete knowledge of the distribution of certain species. Because sparse collections may underestimate the true range of a species, projecting occurrence records onto ALIS segments (including 250 m buffers) filled distribution gaps assumed to occur from imperfect detection. The spatial proximity of fish and mussel species to gB applications was calculated in several ways. First, three spatial search criteria (250 m, 1,000 m, and 2,500 m radii) were applied to species distribution records to determine the proportion of records near gB applications at each search criteria. Conversely, buffers (250 m, 1,000 m, and 2,500 m radii) were also placed around gB locations and the proportion of gB locations that contained a species record was calculated (see Figure 2). Secondly, gB applications within 250 m of a given ALIS segment containing fishes or mussels of conservation concern were identified as those potentially influencing the focal species. This method may have selected nearby tributaries without species records but within

250 m of a record. Conversely, ALIS segments greater than 250 m from a record such as lacustrine locations would not be selected, potentially reducing the overall distribution of a species. However, any discrepancies between the true distribution and inferred distribution is expected to be minor for most species and the metric was retained for spatial scoring, described below. Although this approach incorporates gB applications that have occurred downstream of species of conservation concern, the approach was pursued to understand, in broad terms, which species have the greatest proportion of their range in proximity to gB based on patterns of past applications rather than the specific consequences of an individual application site. In certain cases (principally Lake Sturgeon and American Eel [*Anguilla rostrata*]), the method to estimate the range variable overestimated the proportion of the species' range susceptible to gB applications because the majority of offshore distribution records were not projected onto ALIS segments.



**Figure 2. Spatial search criteria used to calculate the number of fish and mussel occurrence records in proximity to Bayluscide applications. Similarly, species occurrence records were buffered to calculate the number of Bayluscide applications occurring in their vicinity. In this example, species occurrence records exist within 1,000 m and 2,500 m of a Bayluscide application site.**

To determine whether gB applications occurred within critical habitat, the geographic boundaries of areas defined as proposed or final critical habitat for fish and mussel species in the Great Lakes basin were mapped. The subset of these areas containing gB applications, including the number of times gB was applied within a critical habitat polygon, were identified for each species. Larval Sea Lamprey occurrences were also mapped and the proportion of fish or mussel-specific ALIS segments occurring within 250 m of a Sea Lamprey ammocoete record was calculated. The co-occurrence of fish and mussel species with larval Sea Lamprey was not used in the relative risk assessment but indicates the level of spatial correspondence between

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larval Sea Lamprey and each fish and mussel species in question. Spatial analyses were performed using ArcGIS 10.4.

### **Spatial Score**

The spatial value,  $R$ , was calculated as the proportion of a species' range susceptible to gB applications based on the spatial distribution of applications from 2011 - 2017. The calculation was:

$$R = \frac{\text{Number of ALIS segments for species } i \text{ within 250 m of gB application site}}{\text{Total number of ALIS segments for species } i}$$

Although ALIS segments vary by length, patterns in ALIS segment lengths are not thought to be substantially different across species, thereby retaining relativity across species. The spatial value was incorporated because when all else is equal, a greater fraction of the species' range containing gB applications will result in a higher number of individual organisms exposed. In some cases, buffering rules led to the erroneous inclusion or exclusion of ALIS segments. For example, in some cases, ALIS segments within 250 m of gB sites occurred in different watercourses than species occurrence records, usually due to the presence of nearby tributaries undergoing gB applications. In other cases, gB applications were erroneously omitted from assignment to species-specific ALIS segments due to the width of the largest rivers. These issues were manually evaluated and corrected within ArcMap for each species where gB applications were included within large rivers by projecting the application location onto the adjacent ALIS segment and by excluding gB applications occurring in separate watercourses. Offshore records, however, were not included within the distribution identified using ALIS segments. Therefore, species with substantial offshore distributions such as American Eel and Lake Sturgeon had underestimated spatial distribution leading to inflated spatial values when the tributary portion of their range was in close proximity to gB.

### **Intensity Score**

The intensity value,  $I$ , was calculated to quantify the frequency of gB applications occurring within a species' range from 2011–2017. The intensity score was calculated by taking the average gB application effort (i.e., the number of gB applications within species ALIS segments divided by the number of species-specific ALIS segments that contain a gB application) and dividing it by an application threshold. The average gB application effort allowed the comparison of species that were exposed to infrequent gB applications (a low application effort value) with species that were exposed to frequent gB applications (a high application effort value). The gB application threshold was incorporated to normalize gB application effort across the Great Lakes basin from 2011–2017. The application threshold was the product of the 90<sup>th</sup> percentile of applications per assessment in a given year (e.g., six applications per assessment) that had occurred within an ALIS segment from 2011–2017 and the 90<sup>th</sup> percentile of the frequency of gB assessments within an ALIS segment (e.g., four assessments within 2011–2017) from 2011–2017. The intensity value for each species was normalized against the gB application threshold and for cases where the average gB application effort was greater than the gB application threshold, a value of 1.0 was given.

The formulas used to calculate the intensity value,  $I$ , are provided below:

$$I = \begin{cases} \frac{A_B}{T_B}, & A_B < T_B \\ 1, & A_B \geq T_B \end{cases}$$

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$$A_B = \frac{n_{Bi}}{n_{Ai}}$$

Where  $T_B$  is the gB application threshold,  $A_B$  is the average gB application effort,  $n_{Bi}$  is the number of gB applications within species'  $i$  ALIS segments, and  $n_{Ai}$  is the number of ALIS segments of species  $i$  that contains a gB application.

### Habitat Association Score

To describe the habitat associations,  $H$ , that may predispose species to occur within gB application sites, the proportion of occurrence records of fish and mussel species of conservation concern in habitats that would be defined as either Type I or Type II (as opposed to Type III) substrate was calculated as:

$$H = \frac{\text{Number of occurrence records of species } i \text{ within Type I or Type II substrate}}{\text{Total number of occurrence records of species } i \text{ with substrate data}}$$

The three Sea Lamprey program habitat types are defined as follows: Type I habitat is nursery habitat preferred by Sea Lamprey that is composed of fine particle substrate, usually dominated by silt, but may also contain some fine sand and detritus; Type II habitat is nursery habitat acceptable to Sea Lamprey that is composed of coarser substrates relative to Type I including coarse sand, some silt and detritus, and little gravel; and, Type III is habitat not utilized for burying as it is composed of hard and very coarse substrate. To inform habitat associations, all occurrence records where fish ( $n = 4,024$ ) and mussel ( $n = 2,500$ ) species of conservation concern were detected and substrate measurements were taken were compiled from the DFO Biodiversity Database and the DFO Mussel Database (DFO unpublished data). For mussels, both timed-search and quadrat sampling data were used to assess habitat attributes. For each species, the substrate of a site was classified based on the substrate decision tree in Smyth and Drake (2021) (Figure 3). The variable  $H$  reflects the relative propensity for gB applications to occur within sites that contain species of conservation concern based on substrate features, should applications occur within the species' range. The approach to derive  $H$  does not take into account aspects of species rarity, unlike the likelihood of occurrence component of Smyth and Drake (2021). Therefore, habitat results between this paper and Smyth and Drake (2021) are not directly comparable.

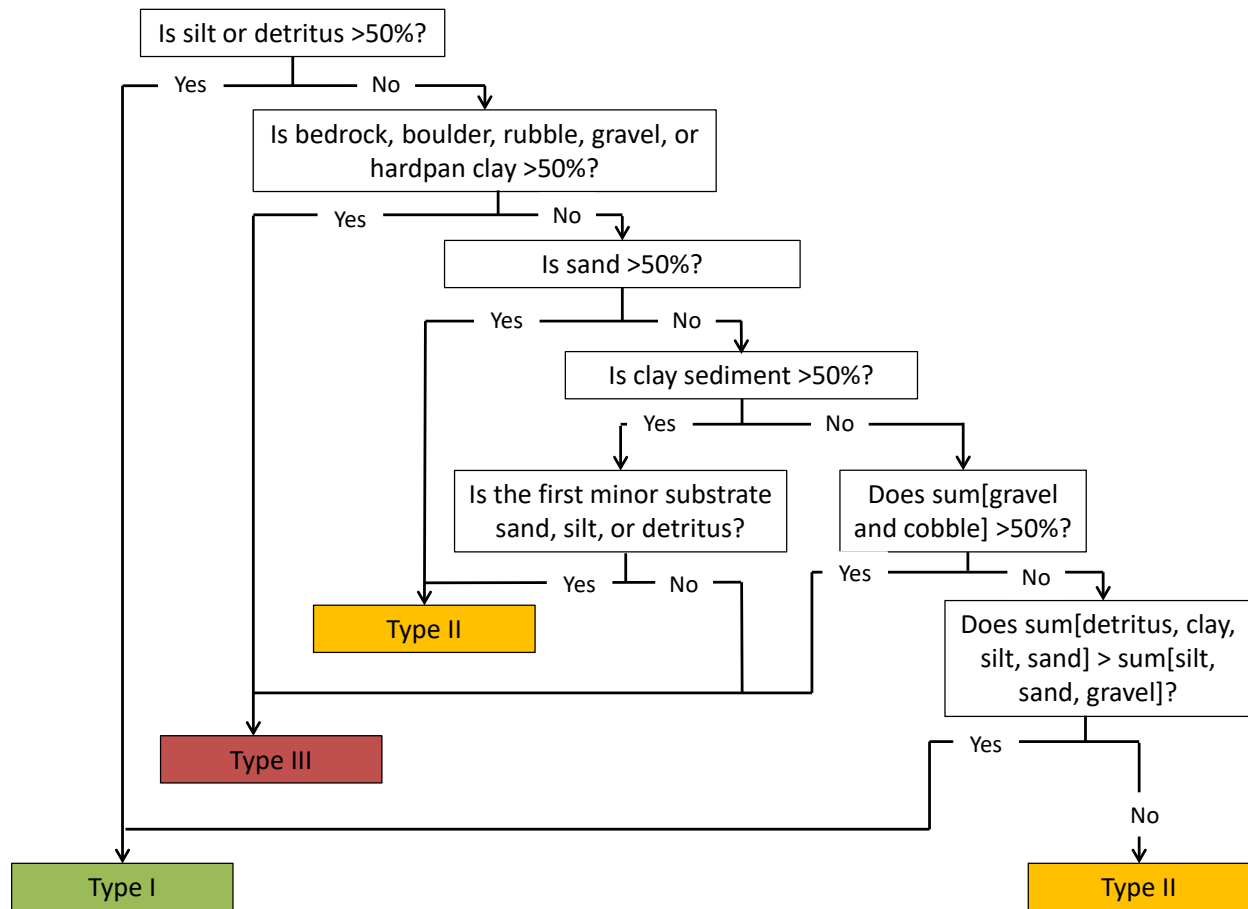


Figure 3. Decision tree used to assign Sea Lamprey habitat classes (Type I, Type II, Type III) based on substrate composition (Reproduced from Smyth and Drake 2021).

Because most sites where native lampreys were captured had a paucity of substrate data, the habitat association value for *Ichthyomyzon* spp. was assumed to be 1.0, based on the presumption that native lamprey habitat associations would match those of Sea Lamprey.

The habitat association value of Lake Sturgeon was based on literature values. The DFO Biodiversity Database is a comprehensive dataset containing the results of targeted, random, and convenience sampling to support various research objectives throughout the Great Lakes basin, but most sampling contained is not sufficient to detect juvenile or adult Lake Sturgeon. Therefore, substrate use from Daugherty et al. (2009) and Gerig et al. (2011) were used. Daugherty et al. (2009) reported a substrate habitat suitability index (HSI) where the sum of HSI values for substrates in Type I or Type II habitat (i.e., clay, silt, and sand) was divided by the sum of HSI values for all substrates. Gerig et al. (2011) reported on the proportion of Lake Sturgeon found at each substrate type for two years. In that study, the sum of proportion values for substrate in Type I or Type II habitat (i.e., clay, silt, sand, and macrophytes) for each year was generated. An overall habitat association value for Lake Sturgeon was generated for this study by taking the average of the three generated proportion values from both studies.

### Toxicity Score

The toxicity value, *T*, was based on the potential mortality rates for fish and mussel species of conservation concern developed in Smyth and Drake (2021; see Table 1). In Smyth and Drake



(2021), four potential mortality rates were calculated for each species based on two different assumed Bayluscide concentrations and two different dose-response curves. To simplify the assignment of toxicity values in this document, only the gentle slope, high concentration (0.057 mg/L; LC<sub>99.9</sub> of Sea Lamprey over eight hour exposure for all non-lamprey species) scenario for each species was considered (see Smyth and Drake 2021 for details). Smyth and Drake (2021) assigned toxicity values to each species of conservation concern based on surrogate species through genus or family-level matches. For several species (Blackstripe Topminnow [*Fundulus notatus*], American Eel, Lake Sturgeon, and Grass Pickerel [*Esox americanus vermiculatus*]), toxicity information for the closest surrogate was beyond the order-level. For these species except American Eel, a surrogate was chosen based on the similarity of life history or habitat. For American Eel, the lack of toxicity information for an appropriate surrogate justified the use of the species most sensitive to Bayluscide, Rainbow Trout (*Oncorhynchus mykiss*). There are a number of reasons why chosen surrogates may poorly reflect the sensitivity of a given fish species (see Smyth and Drake 2021). For this reason, sensitivity analysis was conducted to determine how shifting the assigned toxicity value to the lowest or highest value of any available surrogate would lead to changes in  $RR_M$ . These upper and lower score bounds were also presented visually as error bars relative to assumed surrogate values (see Results of Relative Risk Assessment section).

Table 1. Bayluscide toxicity values for fish and mussel species and surrogates used in the relative risk assessment.

Species of Conservation Concern Common Name	Species of Conservation Concern Scientific Name	Surrogate Species Common Name	Surrogate Species Scientific Name	% Mortality after 8 hour exposure to 0.057 mg/L <sup>A</sup>	% Mortality after 8 hour exposure to 11 mg/L <sup>B</sup>
American Eel	<i>Anguilla rostrata</i>	Rainbow Trout	<i>Oncorhynchus mykiss</i>	63.2	-
Blackstripe Topminnow	<i>Fundulus notatus</i>	Fathead Minnow	<i>Pimephales promelas</i>	3.5	-
Black Redhorse	<i>Moxostoma duquesnei</i>	White Sucker	<i>Catostomus commersonii</i>	13.9	-
Bridle Shiner	<i>Notropis bifrenatus</i>	Fathead Minnow	<i>Pimephales promelas</i>	3.5	-
Channel Darter	<i>Percina copelandi</i>	Yellow Perch	<i>Perca flavescens</i>	4.6	-
Cutlip Minnow	<i>Exoglossum maxillingua</i>	Fathead Minnow	<i>Pimephales promelas</i>	3.5	-
Eastern Sand Darter	<i>Ammocrypta pellucida</i>	Yellow Perch	<i>Perca flavescens</i>	4.6	-
Grass Pickerel	<i>Esox americanus vermiculatus</i>	Yellow Perch	<i>Perca flavescens</i>	4.6	-
Lake Chubsucker	<i>Erimyzon sucetta</i>	White Sucker	<i>Catostomus commersonii</i>	13.9	-

Species of Conservation Concern Common Name	Species of Conservation Concern Scientific Name	Surrogate Species Common Name	Surrogate Species Scientific Name	% Mortality after 8 hour exposure to 0.057 mg/L <sup>A</sup>	% Mortality after 8 hour exposure to 11 mg/L <sup>B</sup>
Lake Sturgeon	<i>Acipenser fulvescens</i>	Channel Catfish	<i>Ictalurus punctatus</i>	53.2	-
Northern Brook Lamprey	<i>Ichthyomyzon fossor</i>	Sea Lamprey	<i>Petromyzon marinus</i>	97.2	-
Northern Madtom	<i>Noturus stigmosus</i>	Channel Catfish	<i>Ictalurus punctatus</i>	53.2	-
Northern Sunfish	<i>Lepomis peltastes</i>	Bluegill	<i>Lepomis macrochirus</i>	7.6	-
Pugnose Minnow	<i>Opsopoeodus emiliae</i>	Fathead Minnow	<i>Pimephales promelas</i>	3.5	-
Pugnose Shiner	<i>Notropis anogenus</i>	Fathead Minnow	<i>Pimephales promelas</i>	3.5	-
Redside Dace	<i>Clinostomus elongatus</i>	Fathead Minnow	<i>Pimephales promelas</i>	3.5	-
River Darter	<i>Percina shumardi</i>	Yellow Perch	<i>Perca flavescens</i>	4.6	-
River Redhorse	<i>Moxostoma carinatum</i>	White Sucker	<i>Catostomus commersonii</i>	13.9	-
Silver Chub	<i>Macrhybopsis storeriana</i>	Fathead Minnow	<i>Pimephales promelas</i>	3.5	-
Silver Lamprey	<i>Ichthyomyzon unicuspis</i>	Sea Lamprey	<i>Petromyzon marinus</i>	97.2	-
Silver Shiner	<i>Notropis photogenis</i>	Fathead Minnow	<i>Pimephales promelas</i>	3.5	-
Spotted Gar	<i>Lepisosteus oculatus</i>	Yellow Perch	<i>Perca flavescens</i>	4.6	-
Spotted Sucker	<i>Minytrema melanops</i>	White Sucker	<i>Catostomus commersonii</i>	13.9	-
Warmouth	<i>Lepomis gulosus</i>	Bluegill	<i>Lepomis macrochirus</i>	7.6	-
Eastern Pondmussel	<i>Ligumia nasuta</i>	-	-	-	15.5
Fawnsfoot	<i>Truncilla donaciformis</i>	Kidneyshell	<i>Ptychobranthus fasciolaris</i>	-	54.3

Species of Conservation Concern Common Name	Species of Conservation Concern Scientific Name	Surrogate Species Common Name	Surrogate Species Scientific Name	% Mortality after 8 hour exposure to 0.057 mg/L <sup>A</sup>	% Mortality after 8 hour exposure to 11 mg/L <sup>B</sup>
Hickorynut	<i>Obovaria olivaria</i>	-	-	-	23.3
Kidneyshell	<i>Ptychobranchnus fasciolaris</i>	-	-	-	54.3
Lilliput	<i>Toxolasma parvum</i>	Kidneyshell	<i>Ptychobranchnus fasciolaris</i>	-	54.3
Mapleleaf	<i>Quadrula quadrula</i>	-	-	-	3.3
Northern Riffleshell	<i>Epioblasma rangiana</i>	Kidneyshell	<i>Ptychobranchnus fasciolaris</i>	-	54.3
Rainbow <sup>C</sup>	<i>Villosa iris</i>	-	-	-	38.3
Rayed Bean <sup>D</sup>	<i>Villosa fabalis</i>	Kidneyshell	<i>Ptychobranchnus fasciolaris</i>	-	54.3
Round Hickorynut	<i>Obovaria subrotunda</i>	-	-	-	44.4
Round Pigtoe	<i>Pleurobema sintoxia</i>	-	-	-	22.4
Salamander Mussel	<i>Simpsonaias ambigua</i>	Kidneyshell	<i>Ptychobranchnus fasciolaris</i>	-	54.3
Snuffbox	<i>Epioblasma triquetra</i>	Kidneyshell	<i>Ptychobranchnus fasciolaris</i>	-	54.3
Threehorn Wartyback	<i>Obliquaria reflexa</i>	Kidneyshell	<i>Ptychobranchnus fasciolaris</i>	-	54.3
Wavyrayed Lampmussel	<i>Lampsilis fasciola</i>	-	-	-	50.8

<sup>A</sup> Values taken from Smyth and Drake (2021). Toxicity for native lamprey species was based on exposure to gB over nine hours

<sup>B</sup> Percent (%) mortality given for mussel species in Newton et al. (2017). Where both sub-adult and adult mortality was given for a species, the higher value was used in the risk assessment.

<sup>C</sup> Scientific name recently revised to *Cambarunio iris*, but *Villosa iris* used here for consistency with SARA listing.

<sup>D</sup> Scientific name recently revised to *Paetulunio fabalis*, but *Villosa fabalis* used here for consistency with SARA listing.

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The toxicity value for mussels was calculated differently with mortality rates for  $T$  taken directly from Newton et al. (2017). Where both sub-adult and adult mortality was given for a species, the higher mortality value was used in the relative risk assessment. The implications of this alternative approach for estimating mortality are explored in Smyth and Drake (2021). Due to differences in how toxicity has been incorporated between fishes and mussels, direct comparison of  $T$  and  $RR_M$  between fishes and mussels is not possible.

No attempt was made to differentiate the values of  $R$ ,  $I$ ,  $H$ ,  $T$ , or  $RR_M$  within species based on factors like age, sex, or behavioural attributes such as the positioning of each species in the water column.

### **Avoidance Behaviour**

The risk assessment incorporates four metrics that are assumed to influence the relative risk of direct mortality for fishes and mussels. However, the assessment does not account for differences in behaviour within or among fish species that may cause some species to detect and leave a site following gB application. Because relatively little is known about fish and mussel avoidance behaviour (but see Boogaard et al. 2016b and Newton et al. 2017) including the concentrations needed to trigger a response, distances moved by fishes or mussels following the detection of gB, or the consequences of relocation (if any), avoidance was not included as a variable in the relative risk assessment. Due to their fossorial nature, mussels do not have the ability to leave a habitat patch once gB is detected. However, they have the ability to close their valves in response to a negative stimulus in the water column. Although valve closure was not included directly within the relative risk assessment equation, valve closure was inherent in the Newton et al. (2017) mortality values used for the toxicity variable.

### **Results of Relative Risk Assessment**

The results of spatial analyses indicated that fish and mussel species differed in the proportion of their range overlapped by gB applications (Table 2). Generally, spatial overlap was relatively insensitive to buffer size. Spatial analyses indicated that gB applications have occurred within areas identified as critical habitat during some point in the study period, including critical habitat for six SARA-listed fish species and 10 SARA-listed mussel species (Table 3, Figure 4, Figure 5). Species in proximity to gB applications were likely to co-occur with Sea Lamprey (Figure 4, Figure 5), though this was not always the case, indicating that gB applications near species of conservation concern do not necessarily detect Sea Lamprey (e.g., Blackstripe Topminnow, River Darter [*Percina shumardi*]; Figure 4) or that Sea Lamprey detections near species of conservation concern are not always associated with gB applications as in when conventional Sea Lamprey assessment methods (e.g., electrofishing) are used (e.g., Redside Dace [*Clinostomus elongatus*]; Figure 4).

Table 2. Proportion of fish or mussel distribution (250 m buffered ALIS segments) containing granular Bayluscide (gB) applications as well as the proportion of gB applications occurring within the range (also 250 m buffered ALIS segments) of fish and mussel species.

Species	Proportion of fish or mussel distribution containing gB applications	Proportion of gB applications within SAR distribution
American Eel	0.088	0.064
Black Redhorse	0.040	0.016
Blackstripe Topminnow	0.056	0.008
Bridle Shiner	0.014	0.002
Channel Darter	0.148	0.102
Cutlip Minnow	0.000	0.000
Eastern Sand Darter	0.017	0.005
Grass Pickerel	0.031	0.021
Lake Chubsucker	0.020	0.005
Lake Sturgeon	0.261	0.043
Northern Brook Lamprey	0.097	0.063
Northern Brook Lamprey + <i>Ichthyomyzon</i> sp.	0.185	0.220
Northern Madtom	0.216	0.120
Northern Sunfish	0.077	0.046
Pugnose Minnow	0.143	0.006
Pugnose Shiner	0.024	0.030
Redside Dace	0.000	0.000
River Darter	0.222	0.003
River Redhorse	0.122	0.032
Silver Chub	0.000	0.000
Silver Lamprey	0.246	0.163

<b>Species</b>	<b>Proportion of fish or mussel distribution containing gB applications</b>	<b>Proportion of gB applications within SAR distribution</b>
Silver Lamprey + <i>Ichthyomyzon</i> sp.	0.251	0.284
Silver Shiner	0.018	0.002
Spotted Gar	0.048	0.003
Spotted Sucker	0.174	0.102
Warmouth	0.039	0.003
Eastern Pondmussel	0.045	0.018
Fawnsfoot	0.133	0.005
Hickorynut	0.167	0.002
Lilliput	0.067	0.003
Kidneyshell	0.023	0.001
Mapleleaf	0.036	0.006
Northern Riffleshell	0.031	0.002
Rainbow	0.012	0.023
Rayed Bean	0.043	0.002
Round Hickorynut	0.077	0.002
Round Pigtoe	0.016	0.001
Salamander Mussel	0.167	0.002
Snuffbox	0.033	0.002
Threehorn Wartyback	0.130	0.004
Wavyrayed Lampmussel	0.016	0.004

Table 3. Number of Bayluscide applications that have occurred in areas that contain critical habitat\* of fishes and mussels in the Canadian waters of the Great Lakes basin.

Species	Critical Habitat* Status	Number of Bayluscide Applications	Application Year(s)
Channel Darter	Final	30	2012, 2014–2017
Eastern Sand Darter	Final	15	2011–2012
Lake Chubsucker	Final	6	2011
Northern Madtom	Final	5	2012–2013
Pugnose Shiner	Final	10	2011
Spotted Gar	Final	5	2011
Eastern Pondmussel	Previously Proposed	4	2014
Kidneyshell	Final	5	2012
Mapleleaf	Previously Proposed	14	2012 and 2014
Northern Riffleshell	Final	1	2012
Rainbow	Previously Proposed	13	2012, 2014–2015, 2017
Rayed Bean	Final	1	2012
Round Hickorynut	Final	4	2012
Round Pigtoe	Final	1	2012
Salamander mussel	Final	1	2012
Snuffbox	Final	1	2012

\* Critical habitat is defined as areas that were considered to be critical habitat during some point in the study period. 'Final' indicates that the critical habitat was identified in a finalized recovery strategy for the species.

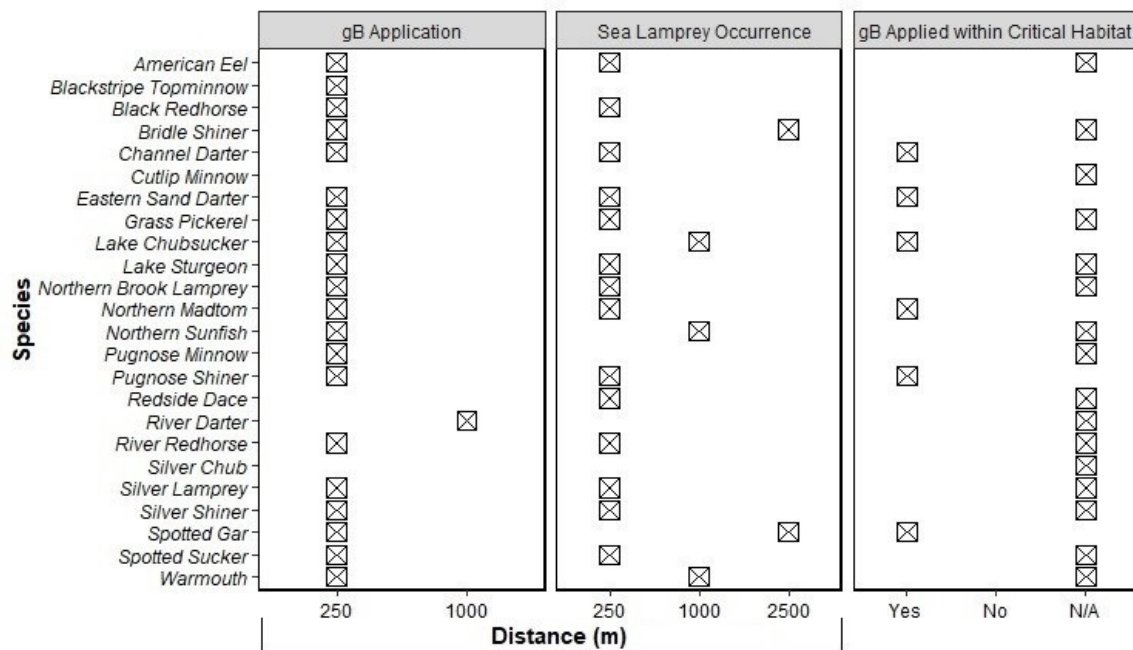


Figure 4. Proximity of nearest fish species occurrence record to granular Bayluscide (gB) applications (left panel) and larval Sea Lamprey occurrence records (middle panel) according to smallest relevant buffer size as well as species where gB applications have occurred within critical habitat (right panel). A value of 'N/A' in the right panel indicates that critical habitat has not been posted to the Species at Risk Public Registry as proposed or finalized for that species while 'No' indicates that critical habitat exists but gB applications have not occurred within the critical habitat area. A 'Yes' indicates that gB has been applied in areas that have contained critical habitat at some point during the study period.

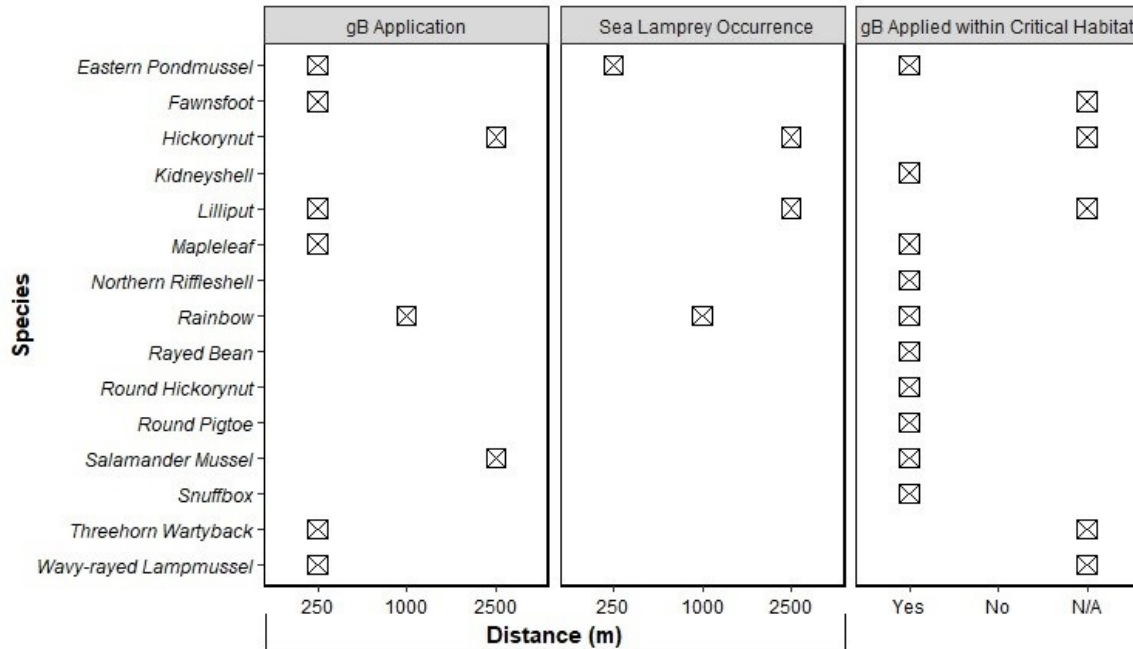


Figure 5. Proximity of nearest mussel species occurrence records to granular Bayluscide (gB) applications (left panel) and larval Sea Lamprey occurrence records (middle panel) according to smallest relevant buffer size as well as species where gB applications have occurred within critical habitat (right panel). A value of 'N/A' in the right panel indicates that critical habitat has not been posted to the Species at Risk Public Registry as proposed or finalized for that species while 'No' indicates that critical habitat exists but gB applications have not occurred within the critical habitat area. A 'Yes' indicates that gB has been applied in areas that have contained critical habitat at some point during the study period.

The values of  $R$ ,  $I$ ,  $H$ ,  $T$ , and  $RR_M$  varied across fishes and mussels (Figures 6–9; see Appendix 3 and 4 for raw values). Highest values of  $R$  (range overlap) for fishes were Lake Sturgeon (0.261), Silver Lamprey (including unidentified *Ichthyomyzon* sp. [0.251]), Silver Lamprey (positive species identification only [0.246]), and River Darter (0.222), and for mussels were Salamander Mussel and Hickorynut (0.167), Fawnsfoot (*Truncilla donaciformis*; 0.133), and Threehorn Wartyback (*Obliquaria reflexa*; 0.13), indicating that the maximum proportion of the range overlapped by gB applications was < 27% for both fishes and mussels but in many cases was much less (Table 2). Further spatial analyses identified the proportion of gB sites within the range of species of conservation concern, which was greatest for Silver Lamprey (+ unidentified *Ichthyomyzon* sp. [0.284]), and Northern Brook Lamprey (+ unidentified *Ichthyomyzon* sp. [0.220]), and for Rainbow (*Cambarunio iris*; 0.023) and Eastern Pondmussel (*Ligumia nasuta*; 0.018), indicating that a considerable fraction of gB applications can be in proximity to a species of conservation concern (Table 2). Intensity values,  $I$ , were highest for Channel Darter (1.0), Northern Brook Lamprey (0.843), Northern Madtom (0.538), and Black Redhorse (*Moxostoma duquesnei*; 0.517), and for Rainbow (0.733), and Eastern Pondmussel (0.425).



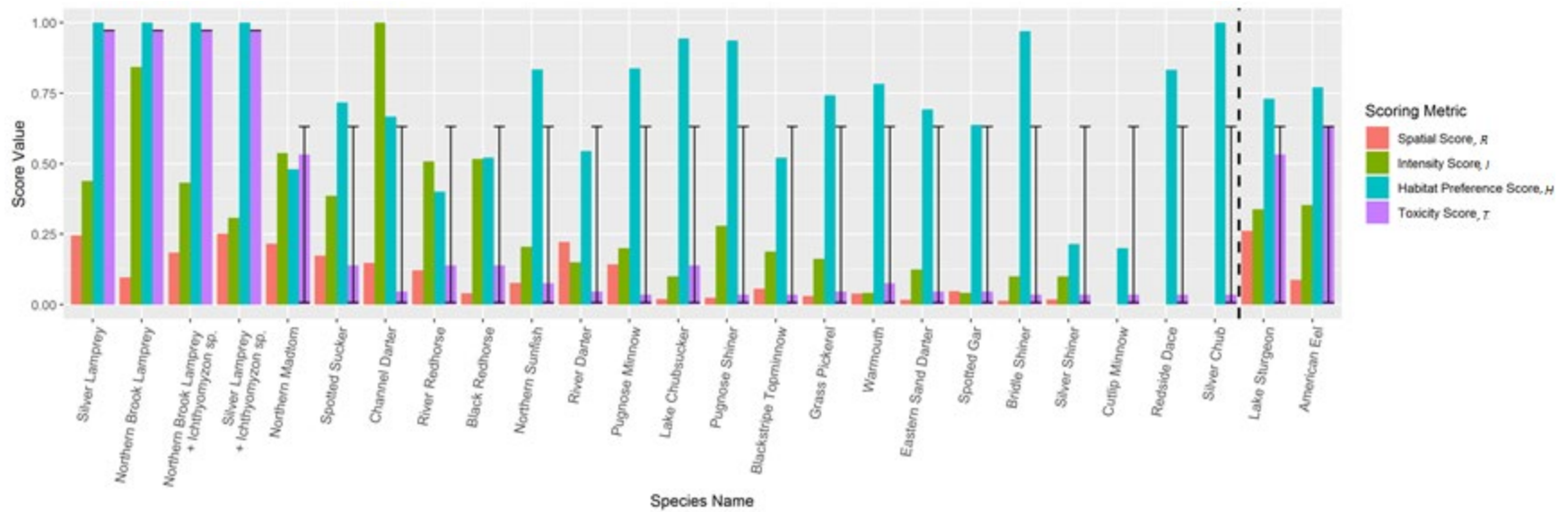


Figure 6. Relative risk assessment metrics ( $R$ ,  $I$ ,  $H$ ,  $T$ ) for fish species of conservation concern. The species order reflects overall relative risk,  $RR_M$ , from highest relative risk on the left to lowest on the right. Error bars on non-lamprey species represent the highest and lowest toxicity value based on all known non-lamprey fish surrogates. Lake Sturgeon and American Eel are separated by a dashed line and are not presented in rank order as their spatial and intensity values are not directly comparable with other fishes.

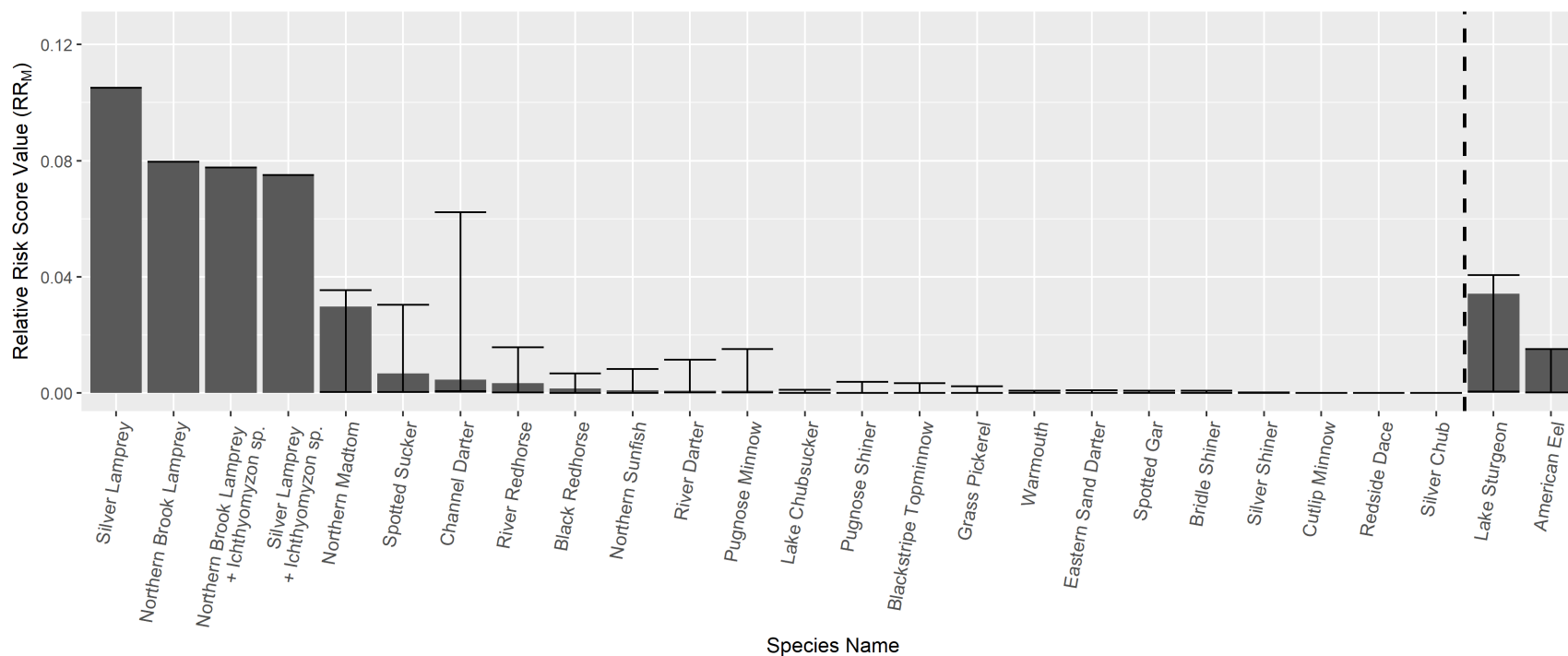


Figure 7. Relative risk,  $RR_M$ , for fish species of conservation concern. Error bars on non-lamprey species represent the highest and lowest possible relative risk using toxicity values from the most sensitive and least sensitive non-lamprey fish surrogates, respectively. Lake Sturgeon and American Eel are separated by a dotted line and not presented in rank order as their relative risk is not directly comparable with other fishes due to assessment methods. Because Silver Lamprey and Northern Brook Lamprey cannot be distinguished as larvae, risk assessment was conducted using only records identified to the species level and with records of *Ichthyomyzon* sp. included. A value of 1 in the y-axis indicates the entire species range is susceptible to granular Bayluscide (gB) applications, applications within the range occur with high intensity, the species occurs only in Type I or Type II habitat, and the species would experience complete mortality given the exposure benchmark. A value of 0 represents no range overlap or intensity, the species occurs only in Type III habitat, and no mortality is expected given the exposure benchmark.

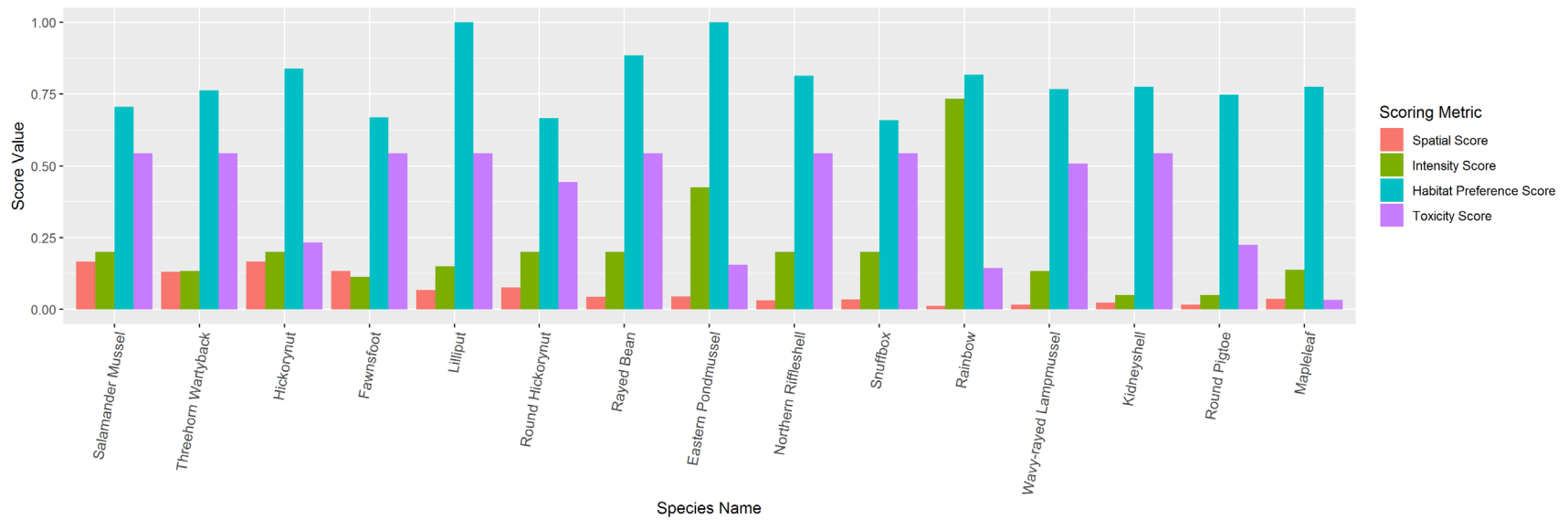


Figure 8. Relative risk assessment metrics (R, I, H, T) for mussel species of conservation concern. The species order reflects the rankings of the relative risk assessment values, from highest relative risk on the left and lowest relative risk on the right.

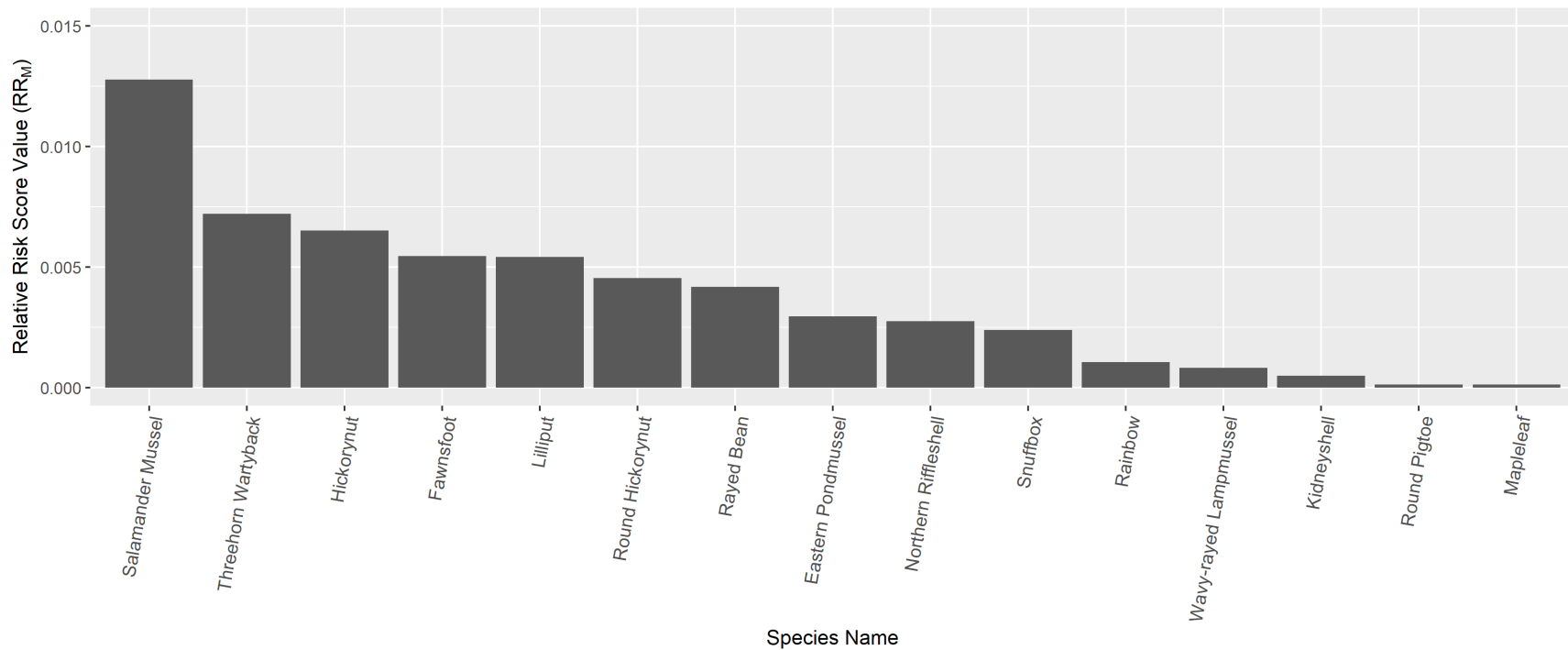


Figure 9. Relative risk,  $RR_M$ , for mussel species of conservation concern. A value of 1 in the y-axis indicates the entire species' range is susceptible to granular Bayluscide (gB) applications, applications within the range occur with high intensity, the species occurs only in Type I or Type II habitat, and the species would experience complete mortality given the exposure benchmark. A value of 0 represents no range overlap or intensity, the species occurs only in Type III habitat, and no mortality is expected given the exposure benchmark.

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Habitat association values,  $H$ , were highest for the four native lamprey species groupings and Silver Chub (*Macrhybopsis storeriana*) [Great Lakes – Upper St. Lawrence populations]; 1.0). Habitat association values were also very high for Bridle Shiner (*Notropis bifrenatus*; 0.970), Lake Chubsucker (*Erimyzon sucetta*; 0.944), and Pugnose Shiner (*Notropis anogenus*; 0.937). For mussels, habitat association values were highest for Eastern Pondmussel (1.0), Lilliput (*Toxolasma parvum*; 1.0), Rayed Bean (*Villosa fabalis*; 0.884), and Hickorynut (0.840). This indicates that for some species, associations with habitats potentially classified as Type I or Type II can be very high, up to 100%. Toxicity values,  $T$ , were highest for native lampreys (0.972), followed by American Eel (0.632), Lake Sturgeon (0.532), and Northern Madtom (0.532), recognizing that each rating was based on surrogate assignments. Error bars provided in Figure 6 represent the highest and lowest toxicity value based on all known fish surrogates. Toxicity for native lamprey species was based on exposure to Bayluscide concentrations of 0.057 mg/L over nine hours. Although all non-lamprey species were based on exposure durations of eight hours, the difference in exposure time is not expected to substantially affect the relative toxicity values (see Smyth and Drake 2021 for clarification). For mussel species, the highest mortality values were approximately 54% at a Bayluscide concentration of 11 mg/L (active ingredient 9.3 mg/L) over eight hours. Many mussel species shared the highest toxicity value (Rayed Bean, Northern Riffleshell [*Epioblasma rangiana*], Lilliput, Kidneyshell [*Ptychobranchus fasciolaris*],  $T = 0.54$ ) due to surrogate assignments.

Most SARA-listed fishes and those assessed by COSEWIC as Endangered, Threatened, or Special Concern exhibited non-zero relative risk values ( $RR_M$ ) (Figure 6 and 7). The overall values of  $RR_M$  resulted in the four native lamprey species groupings having the greatest relative risk of direct mortality for fishes followed by Lake Sturgeon (0.034; noting methodological differences) and Northern Madtom (0.030; Figure 6 and 7). The native lamprey species ranked highly given their habitat use and toxicity values (1.000 and 0.972, respectively), whereas Northern Madtom ranked highly due to high exposure (combined spatial and intensity factors) and toxicity. Lake Sturgeon ranked highly due to high spatial, intensity, and habitat values. However, because offshore lake records of Lake Sturgeon were not incorporated within the ALIS process, spatial and intensity values for Lake Sturgeon are likely inflated, thereby artificially increasing the proportion of the range deemed susceptible to gB applications (similar factors exist for American Eel). Relative risk estimates were sensitive to the surrogate values assumed (Figure 7).

The relative risk assessment for mussels indicated that Salamander Mussel had the greatest relative risk of mortality (0.0128) followed by Threehorn Wartyback (0.0072) and Hickorynut (0.0065). Salamander Mussel and Threehorn Wartyback exhibited high relative risk due to their very high spatial values, reflecting high overlap between past gB applications and the species' range, as well as high toxicity. A high relative risk ranking for Hickorynut was driven by a high habitat association value and high potential spatial overlap with gB applications in comparison to other mussel species.

In the following sections, species accounts provide further information about the distribution and habitat associations of fish and mussel species of conservation concern. Relative risk assessment results are presented for each species and literature concerning direct and indirect pathways of effects is summarized.

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## FISH SPECIES ACCOUNTS

### LAKE STURGEON

**Scientific Name:** *Acipenser fulvescens*

**Designatable Unit (DU):** Great Lakes - Upper St. Lawrence populations

**Current COSEWIC Status and Year of Designation:** Threatened, April 2017

**SARA Schedule, Status and Year of Designation:** No schedule, No status

**Current Committee on the Status of Species at Risk in Ontario (COSSARO) Status and Year of Assessment:** Endangered, November 2017

#### Distribution

The Great Lakes-Upper St. Lawrence Designatable Unit (DU4) occurs in Lake Superior, Lake Huron, Lake Erie, Lake Ontario, the St. Lawrence River, Ottawa River, and their tributaries (COSEWIC 2006a, Golder Associates Ltd. 2011). Distribution in the Great Lakes for records since 1998 is given in Figure A5.1.

#### Allowable Harm

Lake Sturgeon DU4 populations are most sensitive to perturbations that affect young adult survival (89.5 to 154 cm; Vélez-Espino and Koops 2008). From a precautionary perspective, a maximum allowable harm of 1.0–3.7% to adult survival, 1.8–8.2% to juvenile survival, 5.7–13.2% to young of the year (YOY) survival, and 7.1–49.3% to fertility rates has been suggested to avoid jeopardizing the survival and future recovery of Lake Sturgeon. For more information on Lake Sturgeon allowable harm see Vélez-Espino and Koops (2008).

#### Risk Associated with Granular Bayluscide

##### Background

The toxicity of Bayluscide to Lake Sturgeon is not well known. Most toxicity experiments involving Bayluscide have investigated its effects only as an additive to TFM treatments. For example, laboratory toxicity tests conducted in 1988 indicated that exposure of YOY Lake Sturgeon to TFM/1% Bayluscide should be limited to 1.2 times the minimum lethal concentrations during stream treatments (Boogaard et al. 2003). However, agents of the SLCP determined that the reduced lampricide concentration resulted in reduced lampricide treatment effectiveness, which led to the removal of “Protocol for Application of Lampricides to Streams with Populations of Young-of-Year Lake Sturgeon (*Acipenser fulvescens*)” from the Standard Operating Procedures in 2006 (Great Lakes Fishery Commission 2015). Although the specific toxicity of Bayluscide to Lake Sturgeon is unknown, some aspects of Bayluscide-related effects are under investigation. A study by Boogaard et al. (2008b) indicated that juvenile Lake Sturgeon (< 100 mm) displayed avoidance behaviour within four to eight minutes of exposure to granular 3.2% Bayluscide and this behaviour continued for up to 60 minutes. The results of this study were similar to those from a previous study in which juvenile Lake Sturgeon (> 100 mm) showed the ability to avoid gB, demonstrating that juveniles of any size range can detect and avoid gB applications (Bills et al. 2001).

The application of gB may have positive consequences on Lake Sturgeon by reducing Sea Lamprey abundance. Patrick et al. (2009) demonstrated, under laboratory conditions, that Sea Lamprey attacks could cause mortality in sub-adult and adult Lake Sturgeon, either directly by acute anemia after an attack or indirectly from secondary fungal infection. Mortality via both

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pathways was greatest in smaller individuals (450–650 mm). Sea Lamprey control through the application of gB may benefit sub-adult and adult Lake Sturgeon by reducing predation.

### **Relative Risk**

Applications of gB in the Great Lakes basin have high spatial overlap with Lake Sturgeon (Figure 4, Figure A5.2). For example, 26% of all buffered ALIS segments within the range of Lake Sturgeon have experienced at least one gB application from 2011 to 2017 (Table 2) which represents 4% of gB locations. This resulted in a high spatial value in comparison to all other fish species ( $R = 0.261$ ; 91<sup>st</sup> percentile). The intensity of gB applications was also high relative to other fishes ( $I = 0.338$ ; 70<sup>th</sup> percentile).

Lake Sturgeon (adults and juveniles) use of habitat types I and II resulted in a near median value (48<sup>th</sup> percentile) for fishes in this study. Based on the average habitat use in two studies, 73% of Lake Sturgeon occurrences were associated with habitat Types I and II (see Gerig et al. 2011 and Daugherty et al. 2009). This resulted in a moderate habitat preference value ( $H = 0.730$ ; 48<sup>th</sup> percentile) in relation to other fishes. Owing to the lack of specific toxicity information for Lake Sturgeon, Channel Catfish (*Ictalurus punctatus*) was used as a surrogate in the relative risk assessment given similar affinity for benthic habitat (Smyth and Drake 2021), placing the toxicity value for Lake Sturgeon in the top quartile among fishes.

Lake Sturgeon had a high relative risk value ( $RR_M = 0.034$ ; 91<sup>st</sup> percentile; Figure 7), driven largely by high spatial and intensity values (Figure 6). However, direct comparison with other fishes is not possible using current assessment methods given data gaps in coverage where the species is known to be present (e.g. St. Clair River, Detroit River) and the lack of ALIS segment coverage for offshore portions of lakes. These factors may have resulted in inflated spatial and intensity values for Lake Sturgeon relative to other fishes, but indicate non-zero relative risk within the riverine portion of the range.

## **EASTERN SAND DARTER**

**Scientific Name:** *Ammocrypta pellucida*

**Designatable Unit (DU):** Ontario populations

**Current COSEWIC Status and Year of Designation:** Threatened, November 2009

**SARA Schedule, Status and Year of Designation:** Schedule 1, Threatened, June 2003

**Current COSSARO Status and Year of Assessment:** Endangered, November 2009

### **Distribution**

In Canada, Eastern Sand Darter is separated into Ontario and Quebec Designatable Units based on genetic and biogeographical distinctions (COSEWIC 2009). In Ontario, Eastern Sand Darter occurs in tributaries of Lake Erie (Rondeau Bay, Long Point Bay, Grand River, Big Creek) and Lake St. Clair (Detroit River, lower Thames River, and the lower East Sydenham River). It has also recently been collected from the Lake Ontario basin in West Lake (Reid and Dextrase 2014). In Quebec, Eastern Sand Darter occurs in the St. Lawrence River and in its larger tributaries between Lac des Deux Montagnes downstream to Leclercville (COSEWIC 2009). Distribution in the Great Lakes for records since 1998 is provided in Figure A5.3.

### **Allowable Harm**

Eastern Sand Darter populations are most sensitive to perturbations that affect the survival of 0+ individuals and the fertility of 1+ spawners. From a precautionary perspective, a maximum allowable harm of 38% to the annual survival rate of 0+ individuals and 40% to the fertility rate of 1+ spawners has been suggested to avoid jeopardizing the survival and future recovery of

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Canadian populations (Finch et al. 2011). For more information on Eastern Sand Darter allowable harm see Finch et al. (2011).

## **Risk Associated with Granular Bayluscide**

### **Background**

Eastern Sand Darter-specific tolerances to exposure to Bayluscide have not been investigated. However, mortalities of closely related *Etheostoma* spp., such as the Johnny Darter (*E. nigrum*) and Tessellated Darter (*E. olmstedii*), have been observed in the field after the application of Bayluscide. The Fisheries Technical Committee (1999) reported a total of 82 mortalities of Tessellated Darter in three of the five river deltas (Boquet, Ausable, Saranac) treated with Bayer 73 (5% granular) in 1991 and 1995. Two reports by the Great Lakes Fishery Commission (Adair and Sullivan 2013, 2015) also recently reported non-target mortalities of Tessellated Darter (500 individuals) and Johnny Darter (55–65 individuals) after the application of gB in the Ausable River delta in Lake Champlain and Rapid River in Michigan, respectively.

The application of Bayluscide may also impact preferred prey species of Eastern Sand Darter such as midge larvae (Chironomidae) and microcrustaceans. Shiff and Garnett (1961) found that microcrustaceans were markedly reduced in ponds immediately after treatments with 1 mg/L Bayluscide, and Gilderhus (1979) reported a 54% decline in populations of midge larvae seven days after treatment. The microcrustacean populations returned to pre-treatment levels after 32 days whereas midge larvae increased after seven days, but the increase was not significant. Therefore, temporary shortages of preferred prey may occur.

### **Relative Risk**

Approximately 2% of all buffered ALIS segments within the range of Eastern Sand Darter have experienced at least one gB application from 2011 to 2017 (Figure 4, Figure A5.4), which represents only 0.5% of gB applications. This contributed to low spatial ( $R = 0.017$ ; 17<sup>th</sup> percentile among fishes) and intensity values ( $I = 0.125$ ; 35<sup>th</sup> percentile among fishes).

Despite low  $R$  and  $I$  values, 15 gB applications occurred within what is presently Eastern Sand Darter critical habitat from 2011–2012 (Table 3, Figure A5.5). Through the analysis of substrate records, approximately 69% ( $H = 0.693$ ; 39<sup>th</sup> percentile among fishes) of all Eastern Sand Darter collections have occurred within Types I and II habitat. Given the lack of information regarding the sensitivity to gB for this species, the toxicity value in this study was based on potential mortality using information from its closest known surrogate, Yellow Perch (*Perca flavescens*). Based on the published  $LC_{50}$  for Yellow Perch, a mortality of 4.6% at a Bayluscide concentration of 0.057 mg/L over eight hours was used in the risk assessment (Smyth and Drake 2021). This placed the toxicity value for Eastern Sand Darter in the 35<sup>th</sup> percentile among fishes.

The relative risk assessment indicated that risk to Eastern Sand Darter was low in comparison to other fish species of conservation concern ( $RR_M = < 0.001$ ; 26<sup>th</sup> percentile for all fishes; Figure 7).

## **AMERICAN EEL**

**Scientific Name:** *Anguilla rostrata*

**Current COSEWIC Status and Year of Designation:** Threatened, May 2012

**SARA Schedule, Status and Year of Designation:** No schedule, No status

**Current COSSARO Status and Year of Assessment:** Endangered, January 2013



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## Distribution

The historical distribution of American Eel in Canada includes all accessible fresh waters, estuaries, and coastal marine waters from mid-Labrador and the Gulf of St. Lawrence along the Atlantic coast. American Eel can be found within the Ottawa River, St. Lawrence River, and Lake Ontario watersheds. Access to the rest of the Great Lakes (Lake Erie, Huron, and Superior) is the result of stocking and/or dispersal through the Erie and Welland canals (COSEWIC 2012a, Cairns et al. 2014). The distribution of American Eel in freshwater habitats has reduced over the past century, perhaps most significantly in association with the construction of large dams (Chaput et al. 2014). Distribution in the Great Lakes for records since 1998 is provided in Figure A5.6.

## Allowable Harm

Allowable harm for American Eel has not been calculated due to limitations in population modelling associated with lack of abundance data across the seven geographic zones (Young and Koops 2014a).

## Risk Associated with Granular Bayluscide

### Background

The toxicity of Bayluscide to American Eel is not known. However, European Eel (*Anguilla anguilla*), a closely related species, exhibited relatively high mortality to niclosamide at concentrations of 1 mg/L (Buchmann et al. 1990). These results suggest that American Eel may experience directly mortality from gB applications. Unfortunately, data from Buchmann et al. (1990) were collected in a manner that was not conducive for creating a dose-response curve used in Smyth and Drake (2021).

Adult American Eel are benthic omnivores that feed on fishes, molluscs, crustaceans, insect larvae, surface-dwelling insects, worms, and plants (COSEWIC 2012a). The prey species most likely to be adversely affected by Bayluscide application are molluscs and worms, particularly aquatic worms (*Tubifex* spp.), turbellarians, snails (*Physa* spp.; Rye and King 1976), oligochaetes, and midge larvae (Gilderhus 1979). A shift in preferred prey due to a reduction in abundance would most likely adversely affect smaller eels as larger eels feed primarily on fishes and crayfishes (COSEWIC 2012a). Crayfishes have been shown to be relatively resistant to Bayluscide with LC<sub>50</sub> values greater than 50 mg/L (Rye and King 1976).

### Relative Risk

This study found that gB applications have overlapped the known distribution of American Eel (Table 2, Figure A5.7). For example, 9% of all buffered ALIS segments within the range of American Eel have experienced at least one gB application from 2011 to 2017, which represents 0.5% of gB locations. This resulted in spatial and intensity values in the 61<sup>st</sup> and 74<sup>th</sup> percentile among fishes, respectively ( $R = 0.088$ ;  $I = 0.353$ ).

Approximately 77% of all American Eel occurrences have been associated with Types I and II habitat, resulting in a near median habitat preference value (57<sup>th</sup> percentile among fishes). The toxicity value in this study was based on potential mortality using information from its closest known surrogate, Rainbow Trout. Based on the published LC<sub>50</sub> for Rainbow Trout, a mortality of 63% at a Bayluscide concentration of 0.057 mg/L over eight hours was used in the risk assessment (see dose-response curves developed in Smyth and Drake 2021). This placed the toxicity value for American Eel in the top quartile among fishes.

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The risk assessment of gB indicated higher overall relative risk in comparison to many other fishes ( $RR_M = 0.015$ ; 83<sup>rd</sup> percentile; Figure 7), largely driven by spatial, intensity, and toxicity values (Figure 6). However, due to the lack of ALIS segment coverage for lakes and also due to the migratory behaviour of this species, overall gB risk is inflated in comparison to other species. As assessment methods were developed to assess riverine and nearshore lacustrine applications, these factors may have resulted in inflated spatial and intensity values for American Eel and therefore its overall risk value is not directly comparable to other fish species in this study.

## **REDSIDE DACE**

**Scientific Name:** *Clinostomus elongatus*

**Current COSEWIC Status and Year of Designation:** Endangered, November 2017

**SARA Schedule, Status and Year of Designation:** Schedule 1, Endangered, April 2017

**Current COSSARO Status and Year of Assessment:** Endangered, November 2008

### **Distribution**

In Canada, the distribution of Redside Dace is mainly limited to southern Ontario. Most populations occur in tributaries of Lake Ontario from Spencer Creek in the west to Pringle Creek in the east. It is also known from Lake Simcoe (Holland River system), Lake Erie (Irvine Creek), and Lake Huron drainages (Saugeen River system, Gully Creek, Stanley J Tributary, Two Tree River; COSEWIC 2007c, DFO 2019, Lebrun et al. 2020). Distribution in the Great Lakes for records since 1998 is provided in Figure A5.8.

### **Allowable Harm**

Redside Dace populations are most sensitive to perturbations that affect survival of immature individuals (from hatch to age-2) and population-level fecundity (Vélez-Espino and Koops 2009). At a population growth rate of 1.19, allowable harm affecting survival of all age-classes could be only as high as 15% (van der Lee et al. 2020). When population growth rate is below 1, there is no scope for harm. For more information on Redside Dace allowable harm see van der Lee et al. (2020).

## **Risk Associated with Granular Bayluscide**

### **Background**

The tolerance of Redside Dace and closely related species to Bayluscide is unknown but temporary reductions in aquatic prey species may occur. Redside Dace primarily feeds on drifting terrestrial insects, especially adult flies (Diptera) (COSEWIC 2007c), and although toxicity to adult flies has not yet been examined, a reduction of midge larvae populations was reported seven days after exposure to Bayluscide (Gilderhus 1979).

### **Relative Risk**

Recent gB applications have not overlapped with the distribution of Redside Dace. Specifically, zero gB applications since 2011 have occurred within the ALIS segment distribution for Redside Dace (Appendix 1). An analysis of Redside Dace substrate records from DFO's Biodiversity Database found that approximately 83% of records were found within Types I and II habitat. This resulted in a habitat preference value in the 65<sup>th</sup> percentile among fishes. Given that toxicity information for this species is not available, the toxicity value in this study was based on potential mortality using information from its closest known surrogate, Fathead Minnow (*Pimephales promelas*). Based on the published  $LC_{50}$  for Fathead Minnow, a mortality of 3.5%

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at a Bayluscide concentration of 0.057 mg/L over eight hours was used in the risk assessment (Smyth and Drake 2021). This resulted in the lowest toxicity value for Redside Dace and seven other fish species where Fathead Minnow was used as a surrogate.

Overall relative risk ( $RR_M = 0$ ) for Redside Dace was tied for the lowest among fishes (Figure 7), driven by the lack of exposure to gB applications since 2011.

## LAKE CHUBSUCKER

**Scientific Name:** *Erimyzon sucetta*

**Current COSEWIC Status and Year of Designation:** Endangered, November 2008

**SARA Schedule, Status and Year of Designation:** Schedule 1, Endangered, June 2011

**Current COSSARO Status and Year of Assessment:** Threatened, June 2009

### Distribution

In Canada, Lake Chubsucker is restricted to southwestern Ontario. It is found in several wetlands in the Lake Huron, Lake St. Clair, and Lake Erie drainages as well as the tributaries to Lake Erie, Lake St. Clair, and the Niagara River. In Lake Erie, it has been collected from Point Pelee National Park, Rondeau Bay, Long Point Bay, and several tributaries of Big Creek (Staton et al. 2010). Despite sampling efforts, no specimens have been caught from Jeanette's Creek, a tributary of the Thames River, since 1965 (COSEWIC 2008b). Distribution in the Great Lakes for records since 1998 is provided (Figure A5.9).

### Allowable Harm

Lake Chubsucker populations are most sensitive to perturbations that affect survival of immature individuals (from hatch to age-2) and are more sensitive to changes in survival and fecundity of newly mature adults than of older adults (Young and Koops 2011b). From a precautionary perspective, a maximum allowable harm of 33% to juvenile survival (simultaneous harm to ages 0 and 1), 54% to adult survival (ages 2 to 8), and 49% to fecundity of all ages has been suggested to avoid jeopardizing the survival and future recovery of Canadian populations. For more information on Lake Chubsucker allowable harm, see Young and Koops (2011b).

### Risk Associated with Granular Bayluscide

#### Background

To date, there have been no studies documenting the impacts of Bayluscide on Lake Chubsucker. However, Marking and Hogan (1967) reported the toxicity of Bayluscide to 18 freshwater fish species. The species most closely related to Lake Chubsucker was White Sucker (*Catostomus commersonii*), which experienced mortality when exposed to Bayluscide with a  $LC_{50}$  value of 0.084 ppm after 24 hours of exposure (pH 7.5).

Lake Chubsucker feeds on plankton, small crustaceans and molluscs, aquatic insects, and filamentous algae and other plant matter (COSEWIC 2008b). Since Bayluscide was originally developed as a molluscicide, molluscs are extremely sensitive when exposed (24 hour  $LC_{50}$  values < 0.4 mg/L; Rye and King 1976). Shiff and Garnett (1961) also reported a slight overall reduction in microflora in a pond treated with 1 mg/L of Bayluscide, but populations returned to normal after 32 days. As a result of the above factors, if Bayluscide is used in proximity to Lake Chubsucker populations, a temporary shift in preferred prey may occur. Further research is necessary to understand Lake Chubsucker-specific tolerances to exposure to Bayluscide.

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## Relative Risk

This study found that gB applications in the Great Lakes have overlapped with Lake Chubsucker locations in the past (Table 2, Figure 4 and A5.10). For example, approximately 2% of all ALIS segments within the range of Lake Chubsucker have overlapped with a gB application location from 2011–2017 (Table 2), which represents less than 1% of gB locations. This resulted in a low spatial value in comparison to other fishes ( $R = 0.020$ ; 26<sup>th</sup> percentile). Lake Chubsucker also had a low intensity value relative to other fishes in this study (22<sup>nd</sup> percentile).

Approximately 94% of all Lake Chubsucker records were located within areas classified as Type I and II habitat, resulting in a habitat preference value ( $H = 0.944$ ) in the top quartile among fishes. Furthermore, six gB applications occurred within what is presently Lake Chubsucker critical habitat in 2011 (Table 3, Figure A5.11).

Given that toxicity information for this species is not available, the toxicity value in this study was based on potential mortality of a surrogate species (White Sucker). Based on the published  $LC_{50}$  for White Sucker, a mortality of 14% at a Bayluscide concentration of 0.057 mg/L over eight hours was used in the risk assessment (Smyth and Drake 2021), resulting in a toxicity value ( $T = 0.139$ ) in the 65<sup>th</sup> percentile among fishes.

The relative risk assessment found that Lake Chubsucker has moderate relative risk in comparison to other fishes in this study ( $RR_M < 0.001$ ; 48<sup>th</sup> percentile of all fishes; Figure 7). The overall risk value was driven by higher values in the habitat preference and toxicity components of the risk assessment (Figure 6).

## GRASS PICKEREL

**Scientific Name:** *Esox americanus vermiculatus*

**Current COSEWIC Status and Year of Designation:** Special Concern, November 2014

**SARA Schedule, Status and Year of Designation:** Schedule 1, Special Concern, May 2006

**Current COSSARO Status and Year of Assessment:** Special Concern, May 2015

## Distribution

In Canada, Grass Pickerel occurs in southwestern Quebec and southern Ontario. In Ontario, Grass Pickerel is known from wetlands of, and tributaries to, the St. Lawrence River, Lake Ontario, Lake Erie, Lake Huron, Lake St. Clair, and the Severn River watershed (Kahshe Lake, South Kahshe River, Grass Lake). It was caught in the lower Niagara River for the first time in 2014 (COSEWIC 2014a). In Quebec, it has been observed from three sections of the St. Lawrence River and their tributaries in Lake St. Francois, Coteau-du-lac and Lake St. Louis (Beauchamp et al. 2012). Distribution in the Great Lakes basin for records since 1998 is provided in Figure A5.12.

## Risk Associated with Granular Bayluscide

### Background

The effect of Bayluscide on Grass Pickerel and closely related *Esox* species is not well known. However, temporary shortages of preferred prey may occur. Grass Pickerel feeds predominately on fishes and, to a lesser extent, aquatic insects and crustaceans (Beauchamp et al. 2012). Shiff and Garnett (1961) reported a short-term reduction in abundance of microcrustaceans, such as Cladocera and Ostracoda, after treatment with 1 mg/L Bayluscide. Crayfishes appear to be relatively resistant to Bayluscide (Rye and King 1976). A reduction in

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prey fishes, namely those sensitive to the effects of Bayer 73, may also occur (see Marking and Hogan [1967] for toxicity of Bayer 73 to fishes).

### **Relative Risk**

Applications of gB in the Great Lakes basin have overlapped with Grass Pickerel locations in the past (Table 2, Figure A5.13). For example, 3% of all buffered ALIS segments within the range of Grass Pickerel have experienced at least one gB application from 2011 to 2017 (Table 2), which represents 2% of gB locations. This resulted in a moderately low spatial value in comparison to all other fishes ( $R = 0.031$ ; 35<sup>th</sup> percentile). The intensity of applications was moderate relative to other fishes in this study ( $I = 0.163$ ; 43<sup>rd</sup> percentile).

Based on the analysis of substrate records, approximately 74% of all Grass Pickerel have been collected within Type I and II habitat. As a result, Grass Pickerel scored a near median value for fishes for this variable ( $H = 0.742$ ; 52<sup>nd</sup> percentile). Given that toxicity information for this species is not available, the toxicity value in the relative risk assessment was based on potential mortality using information from Yellow Perch. Based on the published  $LC_{50}$  for Yellow Perch, a mortality of 4.6% was assumed at a Bayluscide concentration of 0.057 mg/L over eight hours (Smyth and Drake 2021), resulting in a low toxicity value in comparison to other fishes ( $T = 0.046$ ; 35<sup>th</sup> percentile).

The risk assessment found the overall relative risk for Grass Pickerel to be low in comparison to other fish species ( $RR_M < 0.001$ ; 35<sup>th</sup> percentile overall for fishes; Figure 7), largely due to lower spatial and intensity values.

## **CUTLIP MINNOW**

**Scientific Name:** *Exoglossum maxillingua*

**Current COSEWIC Status and Year of Designation:** Special Concern, November 2013

**SARA Schedule, Status and Year of Designation:** Schedule 1, Special Concern, August 2019

**Current COSSARO Status and Year of Assessment:** Threatened, May 2014

### **Distribution**

In Canada, Cutlip Minnow occurs in the southeastern portion of Ontario in the St. Lawrence River and Ottawa River watersheds. Its range extends from Rivière Saint-Denis in Quebec in the east to Ivy Lea, Ontario in the west and from the lower Ottawa River upstream to Rivière du Diable in the north (COSEWIC 2013a).

## **Risk Associated with Granular Bayluscide**

### **Background**

The tolerance of Cutlip Minnow and closely related species to Bayluscide exposure has not been formally investigated, but the application of Bayluscide granules may result in the mortality of important prey species including chironomids, trichopteran larvae, and oligochaetes. For example, Gilderhus (1979) reported a 54% decline in population of chironomids (midge larvae) and an 80% reduction in oligochaetes seven days after treatment and an elimination of caddisfly population 13 days after treatment of Boardman Lake with Bayer 73 (5% granular formulation). Therefore, a temporary reduction and shift in preferred prey may occur.

### **Relative Risk**

This study found that past gB applications in the Great Lakes have not overlapped with Cutlip Minnow distribution in the past ( $R = 0.00$ ). An analysis of substrate use for this species found

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that only 20% of the records occurred in habitat Types I and II ( $H = 0.200$ ), which was among the lowest habitat value among fishes.

Given that toxicity information for this species is not available, the toxicity value in this study was based on potential mortality using information from its closest known surrogate, the Fathead Minnow. Based on the published  $LC_{50}$  for Fathead Minnow, a mortality of 3.5% at a Bayluscide concentration of 0.057 mg/L over eight hours was assumed (Smyth and Drake 2021). This resulted in the lowest toxicity value for a fish species, tied with seven other species as a result of surrogate choice.

The overall risk to Cutlip Minnow ( $RR_M = 0$ ) was very low in comparison to other fishes (Figure 7), tied for the lowest among fishes due to the presumed lack of exposure to gB since 2011.

## **BLACKSTRIPE TOPMINNOW**

**Scientific Name:** *Fundulus notatus*

**Current COSEWIC Status and Year of Designation:** Special Concern, May 2012

**SARA Schedule, Status and Year of Designation:** Schedule 1, Special Concern, June 2003

**Current COSSARO Status and Year of Assessment:** Special Concern, May 2012

### **Distribution**

In Canada, the distribution of Blackstripe Topminnow is primarily restricted to approximately 500 km<sup>2</sup> of the Sydenham River watershed and nearby tributaries. It has been collected from Bear Creek, Black Creek, East Otter Creek, Fox Creek, Little Bear Creek, Maxwell Creek, Sydenham River, North Sydenham River, West Otter Creek, Whitebread Drain, Plumb Creek, and Nicole Drain (COSEWIC 2012b, DFO unpublished data). Distribution in the Great Lakes for records since 1998 is provided in Figure A5.14.

### **Risk Associated with Granular Bayluscide**

#### **Background**

The effects of Bayluscide on Blackstripe Topminnow has not been investigated in the scientific literature. However, substantial mortality (~ 20,296 individuals) of the closely related Banded Killifish (*Fundulus diaphanus*) was observed after the application of Bayer 73 (5% granular) to five river deltas (Boquet, Ausable, Little Ausable, Salmon, and Saranac) in 1991 and 1995 (Fisheries Technical Committee 1999). In addition, Blackstripe Topminnow appears to be adversely affected when its preferred prey species is unavailable (Gillette 2007). Blackstripe Topminnow is a surface-feeding insectivore that feeds primarily on terrestrial invertebrates entering streams from the riparian zone (COSEWIC 2012b). Gillette (2007) showed that although Blackstripe Topminnow switched food intake to other items when denied access to terrestrial insects, it experienced a reduction in body fat. Therefore, a reduction in terrestrial insects or their aquatic larval form (e.g., midge larvae, caddisfly) after treatment of Bayluscide and temporary shift to other prey items may negatively affect the viability of the species.

#### **Relative Risk**

Applications of gB in the Great Lakes basin have overlapped with Blackstripe Topminnow locations in the past (Table 2, Figure A5.15). Approximately 6% of all ALIS segments within the range of Blackstripe Topminnow have been the subject of gB applications from 2011 to 2017, which represents less than 1% of gB locations. This resulted in a near median spatial value in comparison to other fishes ( $R = 0.056$ ; 52<sup>nd</sup> percentile). The intensity of applications was moderate relative to other fishes in this study ( $I = 0.188$ ; 48<sup>th</sup> percentile).

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Approximately 52% of all Blackstripe Topminnow records were found within Types I and II habitat ( $H = 0.522$ ; 22<sup>nd</sup> percentile). Given that toxicity information for this species is not available, the toxicity value in this study was based on potential mortality using information from the surrogate species, Fathead Minnow. Based on the published  $LC_{50}$  for Fathead Minnow, a mortality of 3.5% at a Bayluscide concentration of 0.057 mg/L over eight hours was assumed ( $T = 0.035$ ; Smyth and Drake 2021), resulting in the lowest toxicity value for fishes (tied with seven other species as a result of surrogate choice).

Overall risk to Blackstripe Topminnow was moderately low ( $RR_M = < 0.001$ ) in comparison with other fishes (Figure 7) due to lower habitat use and toxicity, placing it in the 39<sup>th</sup> percentile for fishes.

## **NORTHERN BROOK LAMPREY**

**Scientific Name:** *Ichthyomyzon fossor*

**Designatable Unit (DU):** Great Lakes - Upper St. Lawrence population

**Current COSEWIC Status and Year of Designation:** Special Concern, April 2007

**SARA Schedule, Status and Year of Designation:** Schedule 1, Special Concern, March 2009

**Current COSSARO Status and Year of Assessment:** Special Concern, November 2008

### **Distribution**

In Canada, Northern Brook Lamprey occurs in Ontario, Quebec, and Manitoba and is comprised of two Designatable Units: the Great Lakes - Upper St. Lawrence population and Saskatchewan - Nelson population. It has been found in tributaries of lakes Superior, Huron, Erie, Ontario, and Nipissing, and the Winnipeg, Ottawa, and St. Lawrence rivers (COSEWIC 2007b). Distribution in the Great Lakes basin for records since 1998 is provided in Figure A5.16.

### **Risk Associated with Granular Bayluscide**

#### **Background**

Scholefield and Seelye (1992) investigated the toxicity to Bayer 73 (70% active ingredient wettable powder formulation) to three genera of larval lampreys (*Ichthyomyzon*, *Lethenteron* [formerly *Lampetra*], and *Petromyzon*) in Lake Huron. They found that  $LC_{99.9}$  values were significantly greater for *Ichthyomyzon* spp. (Northern Brook Lamprey, Silver Lamprey; 70  $\mu\text{g/L}$ ) than for Sea Lamprey (52  $\mu\text{g/L}$ ), but that there was no significant difference for the  $LC_{50}$  values between the Sea Lamprey and *Ichthyomyzon* spp. (36  $\mu\text{g/L}$  and 31  $\mu\text{g/L}$ , respectively). Therefore, treatment with Bayer 73 (wettable powder) is thought to cause similar mortality rates in Sea Lamprey and Northern Brook Lamprey (Scholefield and Seelye 1992). In addition, larval Sea Lamprey assessments conducted using granular 3.2% Bayluscide have resulted in the incidental bycatch of 3,717 *Ichthyomyzon* spp. at 2,720 sites between 1989 and 2006 (Neave *et al.* 2007).

#### **Relative Risk**

This study found that gB applications have overlapped with Northern Brook Lamprey locations in the past (Table 2, Figure A5.17). For example, approximately 10% of all ALIS segments within the range of Northern Brook Lamprey have been overlapped with a gB application location from 2011 to 2017. This value increases to 19% of ALIS segments and 22% of gB locations when unidentified *Ichthyomyzon* sp. records are included, resulting in a high spatial value in comparison to other fishes ( $R = 0.185$ ; 83<sup>rd</sup> percentile). The intensity of applications indicated a high value relative to other fishes in this study ( $I = 0.432$ ; 83<sup>rd</sup> percentile).

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The lack of substrate data prevented an analysis of habitat use in this study, but larval *Ichthyomyzon* spp. have been known to occupy very similar habitats as larval Sea Lamprey given similar burial behaviour in soft sediments. For this reason, individuals belonging to the *Ichthyomyzon* genus were assigned the highest value for the habitat use component of the risk assessment. Based on the published LC<sub>50</sub> for Northern Brook Lamprey, a mortality of 97% at a Bayluscide concentration of 0.057 mg/L over eight hours was assumed (Smyth and Drake 2021), resulting in the highest toxicity value, tied with Silver Lamprey, among fishes.

Overall risk for Northern Brook Lamprey (including unidentified *Ichthyomyzon* sp.) was high in comparison to other fishes ( $RR_M = 0.078$ ; 2<sup>nd</sup> highest value for fishes; Figure 7) due to high values in each of the four components of the risk assessment.

## SILVER LAMPREY

**Scientific Name:** *Ichthyomyzon unicuspis*

**Designatable Unit (DU):** Great Lakes - Upper St. Lawrence population

**Current COSEWIC Status and Year of Designation:** Special Concern, May 2011

**SARA Schedule, Status and Year of Designation:** Schedule 1, Special Concern, August 2019

**Current COSSARO Status and Year of Assessment:** Special Concern, November 2011

### Distribution

In Canada, Silver Lamprey is separated into two Designatable Units: the Great Lakes-Upper St. Lawrence population and the Saskatchewan-Nelson River population. Specimens have been found in the Nelson River drainage of Manitoba, the Great Lakes and their tributaries, and the upper St. Lawrence River and its tributaries (COSEWIC 2011b). New collection records have been found in the Seeber River in the upper Hayes River system which extends the distribution of the Silver Lamprey in northern Manitoba (Tyson and Watkinson 2013). Distribution in the Great Lakes basin for records since 1998 is provided in Figure A5.18.

### Risk Associated with Granular Bayluscide

#### Background

Scholefield and Seelye (1992) investigated the toxicity to Bayer 73 (70% active ingredient wetttable powder formulation) to three genera of larval lampreys (*Ichthyomyzon*, *Lethenteron* [formerly *Lampetra*], and *Petromyzon*) in Lake Huron. They found that the LC<sub>99.9</sub> values were significantly greater for *Ichthyomyzon* spp. (Northern Brook Lamprey, Silver Lamprey; 70 µg/L) than for Sea Lamprey (52 µg/L), but that there was no significant difference for the LC<sub>50</sub> values between Sea Lamprey and *Ichthyomyzon* spp. (36 µg/L and 31 µg/L, respectively). Therefore, treatment with Bayer 73 (wetttable powder) is thought to cause similar mortality rates in Sea Lamprey and Silver Lamprey (Scholefield and Seelye 1992). In addition, Sea Lamprey larval assessments conducted using granular 3.2% Bayluscide resulted in the incidental bycatch of 3,717 *Ichthyomyzon* spp. at 2,720 sites between 1989 and 2006 (Neave et al. 2007).

Of the known host fishes for the parasitic-phase Silver Lamprey, the effects of Bayluscide on Brook Trout (*Salvelinus fontinalis*), Brown Bullhead (*Ameiurus nebulosus*), Common Carp (*Cyprinus carpio*), Goldfish (*Carassius auratus*), Lake Trout, Smallmouth Bass (*Micropterus dolomieu*), White Sucker, and Yellow Perch have been examined. Species most sensitive to Bayluscide after 96 hours of exposure include Brook Trout (LC<sub>50</sub> value of 0.061; Marking and Hogan 1967), Lake Trout (LC<sub>50</sub> value of 0.0494; Bills and Marking 1976), Brown Bullhead (LC<sub>50</sub> value of 0.056; Marking and Hogan 1967), and Smallmouth Bass (LC<sub>50</sub> value of 0.060; Marking



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and Hogan 1967). A reduction in available prey species after Bayluscide application may adversely affect Canadian populations of Silver Lamprey.

### **Relative Risk**

This study found that gB applications in the Great Lakes have overlapped with Silver Lamprey locations in the past (Table 2, Figure A5.19). For example, approximately 25% of all ALIS segments within the range of Silver Lamprey have overlapped with a gB application location from 2011 to 2017, representing 28% of all gB locations. This value does not significantly increase when unidentified *Ichthyomyzon* sp. records are included for this species' distribution, resulting in the 2<sup>nd</sup> highest spatial value for fishes ( $R = 0.185$ ). The intensity of applications for Silver Lamprey (including unidentified *Ichthyomyzon* sp.) was high relative to other fishes in this study ( $I = 0.308$ ; 65<sup>th</sup> percentile; Appendix 1).

The lack of substrate data associated with collection records prevented an analysis of habitat use in this study, but larval *Ichthyomyzon* spp. occupy very similar habitats as larval Sea Lamprey given their similar burial behaviour in soft sediments. For this reason, individuals belonging to the *Ichthyomyzon* genus exhibited the highest value for the habitat use component of the relative risk assessment ( $H = 1.0$ ). Based on the published  $LC_{50}$  for Silver Lamprey, a mortality of 97% at a Bayluscide concentration of 0.057 mg/L over eight hours was used in the risk assessment ( $T = 0.972$ ), resulting in the highest toxicity value, tied with Northern Brook Lamprey, among fishes.

Overall risk for Silver Lamprey (including unidentified *Ichthyomyzon* sp.) was high in comparison to all other fish species ( $RR_M = 0.075$ ; Figure 7). Overall risk for Silver Lamprey excluding unidentified *Ichthyomyzon* sp. was the highest for all fishes in this study ( $RR_M = 0.105$ ). High overall risk was due to high values for each of the four components of the risk assessment equation (Figure 6).

### **SPOTTED GAR**

**Scientific Name:** *Lepisosteus oculatus*

**Current COSEWIC Status and Year of Designation:** Endangered, November 2015

**SARA Schedule, Status and Year of Designation:** Schedule 1, Endangered, August 2019

**Current COSSARO Status and Year of Assessment:** Endangered, June 2016

### **Distribution**

The current range of Spotted Gar in Canada includes coastal wetlands and their tributaries of Lake Erie (Point Pelee National Park, Rondeau Bay, Long Point Bay), and East Lake and Hamilton Harbour in the Lake Ontario drainage (Staton et al. 2012). Distribution in the Great Lakes basin for records since 1998 is provided in Figure A5.20.

### **Allowable Harm**

Spotted Gar populations are most sensitive to perturbations that affect annual survival in early life, and the survival and fertility of early adults. From a precautionary perspective, a maximum allowable harm of 15% to juvenile survival, 19% to age-0 survival, 21% to early adult fertility, and 22% to early adult survival has been suggested to avoid jeopardizing the survival and future recovery of Canadian populations (Young and Koops 2010a). For more information on Spotted Gar allowable harm, see Young and Koops (2010a).

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## Risk Associated with Granular Bayluscide

### Background

No studies have evaluated the toxicity of Bayluscide on Spotted Gar or other closely related *Lepisosteus* spp. Spotted Gar is primarily a piscivorous ambush predator and, in Ontario, Yellow Perch and minnows (Cyprinidae) form a large part of the diet (Scott and Crossman 1973). Marking and Hogan (1967) reported Yellow Perch as being sensitive to Bayer 73 with an LC<sub>50</sub> of 0.081 ppm after 96 hours of exposure (pH of 7.5). Bills and Marking (1976) reported a slightly lower LC<sub>50</sub> value of 0.0639 mg/L after 96 hours of exposure to 70% wettable powder formulation of Bayer 73. Therefore, gB applications may be associated with the decline of the preferred prey of Spotted Gar.

### Relative Risk

This study found that gB applications in the Great Lakes basin have overlapped with Spotted Gar locations (Figure A5.21). Approximately 5% of all ALIS segments within the range of Spotted Gar have overlapped gB application locations (Table 2), which represents less than 1% of all gB applications and resulted in a near median spatial value in comparison with other fishes ( $R = 0.048$ ; 48<sup>th</sup> percentile). The intensity of applications was low relative to other fishes ( $I = 0.042$ ; 13<sup>th</sup> percentile). Despite low intensity, five gB applications occurred within what is presently Spotted Gar critical habitat in 2011 (Table 3, Figure A5.22). Approximately 64% of all Spotted Gar records were found within Types I and II habitat ( $H = 0.637$ ; 30<sup>th</sup> percentile among fishes).

The toxicity value used in this study was based on the surrogate, Yellow Perch, due to similar habitat use as Spotted Gar. Based on the published LC<sub>50</sub> for Yellow Perch, a mortality of 4.6% at a Bayluscide concentration of 0.057 mg/L over eight hours was used in the risk assessment (Smyth and Drake 2021), resulting in a low toxicity value in comparison to other fishes ( $T = 0.046$ ; 35<sup>th</sup> percentile).

Overall risk to Spotted Gar was low in comparison to other fishes ( $RR_M < 0.001$ ; Figure 7; 22<sup>nd</sup> percentile), largely due to lower spatial and intensity values.

## WARMOUTH

**Scientific Name:** *Lepomis gulosus*

**Current COSEWIC Status and Year of Designation:** Endangered, May 2015

**SARA Schedule, Status and Year of Designation:** Schedule 1, Special Concern, June 2003

**Current COSSARO Status and Year of Assessment:** Endangered, January 2016

### Distribution

In Canada, the current and historical distribution of Warmouth is limited to three localities, all situated in the Lake Erie drainage. These locations include Rondeau Bay Provincial Park, Point Pelee National Park (including Hillman Marsh), and Long Point Bay (which includes Big Creek Marsh, Long Point National Wildlife Area, Turkey Point, Crown Marsh, Bluff Bar, and Long Point Inner Bay). Distribution in the Great Lakes for records since 1998 is provided in Figure A5.23.

### Allowable Harm

Warmouth populations are most sensitive to perturbations to the adult life stage. Harm to this portion of the life cycle should be minimized to avoid jeopardizing the survival and recovery of Canadian populations. From a precautionary perspective, chronic annual mortalities of greater than 24.7% to the adult stage or 13.2% to all age-classes is likely to cause population decline,

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assuming a population growth rate of 1.15 (van der Lee and Koops in 2020a). For more information on warmouth allowable harm, van der Lee and Koops (2020a).

## **Risk Associated with Granular Bayluscide**

### **Background**

The impacts of Bayluscide on Warmouth have not been investigated. However, Marking and Hogan (1967) reported the closely related Bluegill (*Lepomis macrochirus*) experienced mortality when exposed to Bayer 73 (LC<sub>50</sub> value of 0.094 ppm after 96 hours at 12°C), with toxicity increasing at higher temperatures (LC<sub>50</sub> value of 0.068 at 17°C). Laboratory experiments have also examined the effects of the 70% wettable powder formulation of Bayer 73, and found Bluegill to be relatively resistant with an LC<sub>50</sub> value of 0.152 mg/L (Bills and Marking 1976). Mortality of Bluegill has also been observed in the field after the application of the 5% granular formulation (Fisheries Technical Committee 1999).

Warmouth feeds on crustaceans, aquatic insects, crayfishes, molluscs, and other fishes (COSEWIC 2015a). Since Bayluscide was originally developed as a molluscicide, molluscs are extremely sensitive when exposed (24 hour LC<sub>50</sub> values < 0.4 mg/L; Rye and King 1976). As a result, a temporary shift in preferred prey may occur. Further research is necessary to understand Warmouth-specific tolerance to Bayluscide exposure.

### **Relative Risk**

Applications of gB in the Great Lakes basin have had minimal overlap with Warmouth (Figure A5.24). For example, 4% of all ALIS segments within the range of Warmouth have overlapped gB application locations from 2011 to 2017, which represents less than 1% of all gB applications. This contributed to low spatial ( $R = 0.039$ ; 39<sup>th</sup> percentile) and intensity values ( $I = 0.042$ ; 13<sup>th</sup> percentile) compared to other fishes in the analysis.

Approximately 78% of all Warmouth records were found within Types I and II habitat, resulting in a moderately high habitat value in relation to other fishes ( $H = 0.783$ ; 61<sup>st</sup> percentile). Using the published LC<sub>50</sub> for Bluegill, a mortality of 7.6% at a Bayluscide concentration of 0.057 mg/L over eight hours was assumed, resulting in a moderate toxicity value ( $T = 0.076$ ; 57<sup>th</sup> percentile).

Overall risk to Warmouth was moderately low ( $RR_M < 0.001$ ; Figure 7) due to low exposure and intensity, placing Warmouth in the 30<sup>th</sup> percentile for fishes.

## **NORTHERN SUNFISH**

**Scientific Name:** *Lepomis peltastes*

**Designatable Unit (DU):** Great Lakes - Upper St. Lawrence population

**Current COSEWIC Status and Year of Designation:** Special Concern, April 2016

**SARA Schedule, Status and Year of Designation:** Schedule 1, Special Concern, August 2019

**Current COSSARO Status and Year of Assessment:** Special Concern, December 2016

### **Distribution**

In Canada, there are two DUs: the Saskatchewan - Nelson River Population and the Great Lakes - Upper St. Lawrence population. The Nelson River Population occurs in northwestern Ontario while the Great Lakes population occurs in southern Ontario and southwestern Quebec. In southern Ontario, Northern Sunfish can be found in tributaries of Lake Huron, Lake Erie, Lake St. Clair, and Lake Ontario, including the Detroit River, Thames River, Sydenham River,

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Ausable River, Saugeen River, Grand River, Maitland River, Trent River, Moira River, Ottawa River, and St. Lawrence River drainages (COSEWIC 2016a). Distribution in the Great Lakes for records since 1998 is provided in Figure A5.25.

## **Risk Associated with Granular Bayluscide**

### **Background**

The impacts of Bayluscide on Northern Sunfish have not been investigated. However, Marking and Hogan (1967) reported the closely related Bluegill to be sensitive to Bayer 73 (LC<sub>50</sub> value of 0.094 ppm after 96 h at 12°C), with toxicity increasing at higher temperatures (LC<sub>50</sub> value of 0.068 at 17°C). Laboratory experiments have also examined the effects of the 70% wettable powder formulation of Bayer 73, and found Bluegill to be relatively resistant with an LC<sub>50</sub> value of 0.152 mg/L (Bills and Marking 1976). Mortality of Bluegill has also been observed in the field after the application of the 5% granular formulation (Fisheries Technical Committee 1999).

Northern Sunfish feeds primarily on insects but also feeds on other invertebrates and sometimes small fishes (Scott and Crossman 1973). Exposure to Bayluscide has caused reductions in abundance of aquatic insects and some crustaceans. For example, Shiff and Garnett (1961) reported a short-term reduction in abundance of microcrustaceans, such as Cladocera and Ostracoda, after treatment with 1 mg/L Bayluscide. Furthermore, a reduction of midge larvae populations was reported seven days after exposure to Bayluscide (Gilderhus 1979). As a result, temporary shifts in prey items may occur.

### **Relative Risk**

This study found that gB applications in the Great Lakes have overlapped with Northern Sunfish locations in the past (Figure A5.26). For example, approximately 8% of all ALIS segments within the range of Northern Sunfish have overlapped with a gB application location from 2011 to 2017 (Table 2), which represents 5% of all gB applications and resulted in moderate spatial ( $R = 0.077$ ; 57<sup>th</sup> percentile) and intensity values ( $I = 0.205$ ; 57<sup>th</sup> percentile) compared to other fishes.

Approximately 83% of all Northern Sunfish records have been located within Types I and II habitat, resulting in a high habitat value for the species ( $H = 0.834$ ; 70<sup>th</sup> percentile). Using the published LC<sub>50</sub> for Bluegill, a mortality of 7.6% at a Bayluscide concentration of 0.057 mg/L over eight hours was used as the toxicity value ( $T = 0.076$ ; 57<sup>th</sup> percentile), was moderate in comparison to other fishes.

Overall risk to Northern Sunfish was moderate ( $RR_M = 0.001$ ; Figure 7), placing Northern Sunfish in the 61<sup>st</sup> percentile for fishes.

## **SILVER CHUB**

**Scientific Name:** *Macrhybopsis storeriana*

**Designatable Unit (DU):** Great Lakes - Upper St. Lawrence population

**Current COSEWIC Status and Year of Designation:** Endangered, May 2012

**SARA Schedule, Status and Year of Designation:** Schedule 1, Endangered, August 2019

**Current COSSARO Status and Year of Assessment:** Threatened, May 2012

### **Distribution**

In Canada, Silver Chub is separated into two DUs: the Great Lakes-Upper St. Lawrence populations and the Saskatchewan-Nelson River populations (COSEWIC 2012e). In the Great Lakes basin, it is limited to Lake Erie, Lake St. Clair, and the southern portion of Lake Huron. It

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has also recently been found in the lower Thames River, which is the first riverine detection in Ontario waters. In Manitoba, it is found in southern Lake Winnipeg and in the Assiniboine and Red River drainages (COSEWIC 2012e).

### **Allowable Harm**

Growing populations of Silver Chub are most sensitive to perturbations that affect fecundity or the survival of YOY (Young and Koops 2013a). However, stable or declining populations are most sensitive to changes in adult survival. When a population trajectory is stable, transient harm (allowable one-time removal, performed no more frequently than every seven years) should not exceed a 15% reduction in adult abundance, or a 23.5% reduction in YOY abundance, or an 8.5% reduction in total abundance to avoid jeopardizing the survival and future recovery of Canadian populations. For more information on Silver Chub allowable harm, see Young and Koops (2013a).

### **Risk Associated with Granular Bayluscide**

#### **Background**

Specific tolerances of Silver Chub to Bayluscide have not been reported but non-target mortality has been observed among closely related *Notropis* spp. Approximately 9,385 Mimic Shiner (*N. volucellus*), 2,168 Spottail Shiner (*N. hudsonius*), and 185 Emerald Shiner (*N. atherinoides*) died after treating five river deltas (Boquet, Ausable, Little Ausable, Salmon, and Saranac) in Lake Champlain with Bayer 73 (5% granular) in 1991 and 1995 (Fisheries Technical Committee 1999).

Silver Chub prey species at greatest risk of mortality after treatment with Bayluscide include molluscs, small crustaceans, and some aquatic insect larvae such as midge larvae (Shiff and Garnett 1961, Rye and King 1976, Gilderhus 1979). However, mayfly nymphs appear relatively resistant to Bayluscide concentrations as high as 0.4 mg/L (Bills et al. 1985).

#### **Relative Risk**

This study found that recent gB applications have not overlapped with the distribution of Silver Chub. Specifically, zero gB applications since 2011 have occurred within the ALIS segment distribution for Silver Chub (Appendix 1); however, approximately 100% of records were found within Types I and II habitat. As a result, Silver Chub scored the highest habitat preference value for fishes, tied with Silver Lamprey and Northern Brook Lamprey.

Given that toxicity information for this species is not available, the toxicity value in this study was based on potential mortality using information from its closest known surrogate, Fathead Minnow. Based on the published LC<sub>50</sub> for Fathead Minnow, a mortality of 3.5% at a Bayluscide concentration of 0.057 mg/L over eight hours was used, resulting in the lowest toxicity value and tied with seven other species as a result of surrogate choice.

The overall risk to Silver Chub was low ( $RR_M = 0$ ; Figure 7), among the lowest for fishes, due to the lack of exposure over the course of the study period.

### **SPOTTED SUCKER**

**Scientific Name:** *Minytrema melanops*

**Current COSEWIC Status and Year of Designation:** Special Concern, November 2014

**SARA Schedule, Status and Year of Designation:** Schedule 1, Special Concern, June 2003

**Current COSSARO Status and Year of Assessment:** Special Concern, May 2015

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## Distribution

In Canada, Spotted Sucker occurs in lakes St. Clair and Erie, including the Detroit, Sydenham, and Thames rivers. It has also been caught in several tributaries of Lake St. Clair, including Maxwell Creek, Bear Creek, Little Bear Creek, and Whitebread Drain (COSEWIC 2014b). Collections in Lake Erie are restricted to the central and western basin, from the mouth of the Detroit River to Rondeau Bay (Edwards and Staton 2009, COSEWIC 2014b). Distribution in the Great Lakes for records since 1998 is provided in Figure A5.27.

## Risk Associated with Granular Bayluscide

### Background

No studies have evaluated the toxicity of Bayluscide on Spotted Sucker. However, Marking and Hogan (1967) reported on the toxicity of a closely related species, White Sucker, which experienced mortality when exposed to Bayluscide, having a  $LC_{50}$  value of 0.084 ppm after 24 h of exposure (pH 7.5). Marking and Bills (1985) also found that when Bayer 73 is paired with other contaminants, including organic pesticides, metal, industrial or municipal pollutants (e.g., DDT, Endrin, malathion, carbaryl, toxaphene, Delnav, cadmium copper, zinc, ammonia, nitrite, cyanide, and chlorine), toxicity effects were additive when exposure occurred for 96 hours. Therefore, application of Bayluscide in areas where pollution occurs may increase the risk of mortality. Mortality of approximately 500 White Sucker was reported after the application of Bayluscide to Rapid River in 2012 (Adair and Sullivan 2013).

Indirect effects for Spotted Sucker resulting from Bayluscide applications are poorly known. The Spotted Sucker feeds on a variety of prey items including diatoms, zooplankton, chironomids, and molluscs (COSEWIC 2005). Chironomids and molluscs are particularly sensitive to exposure to Bayluscide and may experience a reduction in abundance after treatment (Rye and King 1976, Gilderhus 1979), which may lead to food web effects for Spotted Sucker.

### Relative Risk

This study found that gB applications in the Great Lakes have overlapped with Spotted Sucker locations in the past (Figure A5.28). For example, approximately 17% of all ALIS segments within the range of Spotted Sucker have overlapped with gB application locations from 2011 to 2017, which represents approximately 10% of all gB applications (Table 2) and contributed to high spatial ( $R = 0.174$ ; 78<sup>th</sup> percentile) and intensity values ( $I = 0.386$ ; 78<sup>th</sup> percentile).

Approximately 72% of all Spotted Sucker records have been found within Types I and II habitat, resulting in a high habitat value in comparison with other fishes ( $H = 0.717$ ; 78<sup>th</sup> percentile). Given that toxicity for this species is not known, the toxicity value in this study was based on potential mortality in White Sucker. Based on the published  $LC_{50}$  for White Sucker, a mortality of 14% at a Bayluscide concentration of 0.057 mg/L over eight hours was used in the risk assessment ( $T = 0.139$ ; 65<sup>th</sup> percentile), which was moderately high in comparison to other fishes.

Overall risk to Spotted Sucker was moderately high ( $RR_M = 0.0067$ ; Figure 7), placing Spotted Sucker in the 78<sup>th</sup> percentile for fishes. This result was driven by moderately high spatial, intensity, and toxicity values.

## RIVER REDHORSE

**Scientific Name:** *Moxostoma carinatum*

**Current COSEWIC Status and Year of Designation:** Special Concern, November 2015

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**SARA Schedule, Status and Year of Designation:** Schedule 1, Special Concern, December 2007

**Current COSSARO Status and Year of Assessment:** Special Concern, June 2016

## **Distribution**

In Canada, River Redhorse is found in southern and eastern Ontario and southern and southwestern Quebec. In Ontario, it occurs in the lower Thames, Grand River, Trent, Mississippi, Madawaska, and Ottawa rivers and the Bay of Quinte. In Quebec, it occurs in the Coloungé, Gatineau, Noire, and Richelieu river systems. It is presumed extirpated from the Ausable River in Ontario, and the Châteauguay and Yamaska rivers in Quebec (COSEWIC 2006b). Distribution in the Great Lakes for records since 1998 is provided in Figure A5.29.

## **Risk Associated with Granular Bayluscide**

### **Background**

No studies have evaluated the toxicity of Bayluscide on River Redhorse, but Marking and Hogan (1967) reported on the toxicity of a closely related species, White Sucker, which exhibited a  $LC_{50}$  value of 0.084 ppm after 24 h of exposure (pH 7.5). Marking and Bills (1985) found that when Bayer 73 was paired with other contaminants, including organic pesticides, metal, industrial or municipal pollutants (e.g., DDT, Endrin, malathion, carbaryl, toxaphene, Delnav, cadmium copper, zinc, ammonia, nitrite, cyanide, and chlorine), toxicity effects were additive during exposure durations of 96 hours. Therefore, application of Bayluscide in areas where pollution occurs may increase the risk of mortality. Mortality of approximately 500 White Sucker was also reported after the application of Bayluscide to Rapid River in 2012 (Adair and Sullivan 2013).

Bayluscide application may result in local food web changes. River Redhorse feed primarily on benthic invertebrates including molluscs, insect larvae and crayfishes (COSEWIC 2006b). Molluscs and insect larvae including caddisflies and chironomids are particularly sensitive to exposure to Bayluscide and might experience a reduction in abundance after treatment (Rye and King 1976, Gilderhus 1979), which may contribute to the decline of mollusc-feeding catostomids.

### **Relative Risk**

This study found that gB applications in the Great Lakes have overlapped with River Redhorse locations in the past (Figure A5.30). For example, approximately 12% of all ALIS segments within the range of River Redhorse have overlapped with a gB application location from 2011 to 2017 (Table 2), which represents approximately 3% of all gB applications. This contributed to higher spatial ( $R = 0.122$ ; 65<sup>th</sup> percentile) and intensity values ( $I = 0.508$ ; 87<sup>th</sup> percentile) in comparison to other fishes (Figure 6).

Based on the analysis of substrate records, 40% of all River Redhorse records have been collected from Types I and II habitat, resulting in a low habitat value for the species in comparison to other fishes ( $H = 0.40$ ; 9<sup>th</sup> percentile). Given that toxicity information for this species is not available, toxicity was based on potential mortality using its closest known surrogate, White Sucker. Based on the published  $LC_{50}$  for White Sucker (mortality of 14% at a Bayluscide concentration of 0.057 mg/L over eight hours), toxicity was moderately high in comparison to other fishes ( $T = 0.139$ ; 65<sup>th</sup> percentile).

Overall risk to River Redhorse was high in comparison to other fishes ( $RR_M = 0.0035$ ; 70<sup>th</sup> percentile; Figure 7), driven by high toxicity, spatial, and intensity values.

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## BLACK REDHORSE

**Scientific Name:** *Moxostoma duquesnei*

**Current COSEWIC Status and Year of Designation:** Threatened, May 2015

**SARA Schedule, Status and Year of Designation:** Schedule 1, Threatened, August 2019

**Current COSSARO Status and Year of Assessment:** Threatened, January 2016

### Distribution

In Canada, Black Redhorse is found in southern Ontario in tributaries of Lake Huron, and Lake Erie. This includes the Grand River, Ausable River, Saugeen River, Thames River, and Bayfield River. Distribution in the Great Lakes for records since 1998 is provided Figure A5.31.

### Allowable Harm

Black Redhorse populations are most sensitive to perturbations that affect the survival of immature individuals (from hatch to age 4), and early adults (ages 2-8) (Young and Koops 2014b). Harm to these portions of the life cycle should be minimized to avoid jeopardizing the survival and recovery of Canadian populations (Young and Koops 2014b). From a precautionary perspective, a maximum allowable harm for survival rates of YOY, juveniles, and young adults should be less than 19%, 14%, and 13%, respectively (Velez-Espino and Koops 2009). For more information on Black Redhorse allowable harm, see Velez-Espino and Koops (2009).

### Risk Associated with Granular Bayluscide

#### Background

No studies have evaluated the toxicity of Bayluscide on Black Redhorse, but Marking and Hogan (1967) reported on the toxicity of a closely related species, White Sucker, which exhibited a  $LC_{50}$  value of 0.084 ppm after 24 h of exposure (pH 7.5). Marking and Bills (1985) also found that when Bayer 73 was paired with other contaminants, including organic pesticides, metal, industrial or municipal pollutants (e.g., DDT, Endrin, malathion, carbaryl, toxaphene, Delnav, cadmium copper, zinc, ammonia, nitrite, cyanide, and chlorine), toxicity was additive when exposed for 96 hours. Therefore, application of Bayluscide in areas where pollution occurs may increase the risk of mortality. Mortality of approximately 500 White Sucker has been reported after the application of Bayluscide to Rapid River in 2012 (Adair and Sullivan 2013).

Black Redhorse is primarily planktivorous when under 65 mm in length and benthivorous when over 65 mm in length (COSEWIC 2015b). It feeds on benthic invertebrates including crustaceans and insects as well as macrophytes (Coker et al. 2001). Changes in food web dynamics as a result of Bayluscide application has the potential to affect the species. Insect larvae are particularly sensitive to exposure to Bayluscide and might experience a reduction in abundance after treatment (Rye and King 1976, Gilderhus 1979), which has the potential to impact Black Redhorse populations.

#### Relative Risk

This study found that gB applications in the Great Lakes have overlapped with Black Redhorse locations in the past (Table 2; Figure A5.32). For example, from 2011 to 2017, approximately 4% of all ALIS segments within the range of Black Redhorse have overlapped gB application locations, which represents less than 2% of all gB applications. This contributed to a moderate spatial value ( $R = 0.040$ ; 43<sup>rd</sup> percentile; Figure 6) and a high intensity value ( $I = 0.517$ ; 91<sup>st</sup> percentile).



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Approximately 52% of all Black Redhorse records have been found within Types I and II habitat, resulting in a low habitat value ( $H = 0.522$ ; 17<sup>th</sup> percentile). Given that toxicity information for this species was not available, toxicity was based on potential mortality in White Sucker. Based on the published  $LC_{50}$  for White Sucker, a mortality of 14% at a Bayluscide concentration of 0.057 mg/L over eight hours was used, indicating moderate toxicity ( $T = 0.139$ ; 65<sup>th</sup> percentile) in comparison to other fishes.

Overall risk to Black Redhorse was moderately high in comparison to all other fish species ( $RR_M = 0.0015$ ; Figure 7; 65<sup>th</sup> percentile), resulting from high toxicity and intensity values.

## **PUGNOSE SHINER**

**Scientific Name:** *Notropis anogenus*

**Current COSEWIC Status and Year of Designation:** Threatened, May 2013

**SARA Schedule, Status and Year of Designation:** Schedule 1, Threatened, August 2019

**Current COSSARO Status and Year of Assessment:** Threatened, June 2013

### **Distribution**

In Canada, Pugnose Shiner occurs in six disjunct areas in Ontario: the southern Lake Huron drainage, Lake St. Clair and its tributaries, Lake Erie, eastern Lake Ontario, and the St. Lawrence River. In Lake Erie, it historically occurred in Point Pelee National Park and Rondeau Bay, but may now only be present in Long Point Bay and the mouth of the Canard River (COSEWIC 2013b). Distribution in the Great Lakes for records since 1998 is provided in Figure A5.33.

### **Allowable Harm**

Pugnose Shiner populations are most sensitive to perturbations that affect survival in the first two years of life, and the fecundity of first-time spawners. From a precautionary perspective, a maximum allowable harm of 14% to 1 or 2 year-old survival, or 15% to the fertility rate of first time spawners has been suggested to avoid jeopardizing the survival and future recovery of Canadian populations (Venturelli et al. 2010b). For more information on Pugnose Shiner allowable harm, see Venturelli et al. (2010b).

### **Risk Associated with Granular Bayluscide**

#### **Background**

The toxicity of Bayluscide to Pugnose Shiner has not been examined, but several studies have been conducted on a closely related species, Fathead Minnow. Marking and Hogan (1967) reported that Fathead Minnow did experience mortality when exposed to Bayer 73 ( $LC_{50}$  value of 0.106 ppm; pH of 7.5). Marking and Bills (1985) also reported a lethal concentration in Fathead Minnow ( $LC_{50}$  of 0.11 mg/L) when exposed to Bayer 73, but found that when paired with 13 other contaminants including pesticides, heavy metals and industrial pollutants (DDT, Endrin, malathion, carbaryl, toxaphene, Delnav, cadmium copper, zinc, ammonia, nitrite, cyanide, and chlorine) the toxicity was additive when exposed for 96 hours. Therefore, the potential for increased toxicity when combined with other chemicals, especially pollutants, should be considered when applying Bayluscide in areas with closely related species at risk, including Pugnose Shiner.

Given that Pugnose Shiner feeds on plants, algae, small leeches, cladocerans (*Chydorus sphaericus* and *Bosmina longirostris*), and trichopterans (COSEWIC 2013b), it is possible that negative indirect effects on the local food web could impact the species. Leeches (24 h  $LC_{50}$

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< 0.05 mg/L) and caddisflies appear to be very susceptible to the effects of Bayluscide (Gilderhus 1979, Dawson 2003), which may result in a reduction in abundance and temporary shift of prey species.

### **Relative Risk**

This study found that gB applications in the Great Lakes have overlapped with Pugnose Shiner locations in the past (Table 2, Figure A5.34). For example, approximately 2% of all ALIS segments within the range of Pugnose Shiner have overlapped with a gB application location from

2011– 2017 (Table 2), which represents 3% of all gB applications during that period and resulted in a low spatial value ( $R = 0.024$ ; 30<sup>th</sup> percentile) in comparison to other fishes (Figure 6). The species exhibited a moderately high intensity value relative to other fishes ( $I = 0.280$ ; 61<sup>st</sup> percentile). Notably, 10 gB applications occurred within what is presently Pugnose Shiner critical habitat in 2011 (Table 3; Figure A5.35).

Approximately 94% of all Pugnose Shiner records have been collected within Types I and II habitat ( $H = 0.937$ ; 78<sup>th</sup> percentile). Because specific toxicity information for this species is not available, toxicity was based on published  $LC_{50}$  for Fathead Minnow (mortality of 3.5% at a Bayluscide concentration of 0.057 mg/L over eight hours), resulting in the lowest toxicity value among fishes.

Overall risk to Pugnose Shiner was moderate relative to other fish species in this study ( $RR_M < 0.001$ ; 43<sup>rd</sup> percentile; Figure 7).

## **BRIDLE SHINER**

**Scientific Name:** *Notropis bifrenatus*

**Current COSEWIC Status and Year of Designation:** Special Concern, May 2013

**SARA Schedule, Status and Year of Designation:** Schedule 1, Special Concern, 2003

**Current COSSARO Status and Year of Assessment:** Special Concern, June 2013

### **Distribution**

In Canada, Bridle Shiner can be found in tributaries of eastern Lake Ontario and the St. Lawrence River including the Rideau River. In Quebec, it is found in tributaries of the St. Lawrence, Lake Saint-Pierre and Lake Memphrémagog (COSEWIC 2013c). Distribution in the Great Lakes for records since 1998 is provided in Figure A5.36.

## **Risk Associated with Granular Bayluscide**

### **Background**

The tolerance of Bridle Shiner to Bayluscide has not yet been examined, but several studies have been conducted on a closely related species, Fathead Minnow. Marking and Hogan (1967) reported mortality of Fathead Minnow when exposed to Bayer 73 ( $LC_{50}$  value of 0.106 ppm; pH of 7.5). Marking and Bills (1985) also reported a lethal concentration in Fathead Minnow ( $LC_{50}$  of 0.11 mg/L) when exposed to Bayer 73, but found that when paired with 13 other contaminants including pesticides, heavy metals and industrial pollutants (DDT, Endrin, malathion, carbaryl, toxaphene, Delnav, cadmium copper, zinc, ammonia, nitrite, cyanide, and chlorine), toxicity was additive when exposed for 96 hours. Therefore, the potential for increased toxicity when combined with other chemicals, especially pollutants, should be considered when applying Bayluscide in areas with closely related species at risk, including Bridle Shiner.

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Bayluscide application may result in local food web changes. Aquatic insects such as Caddisflies appear to be very susceptible to the effects of Bayluscide (Gilderhus 1979, Dawson 2003), which may result in a reduction of the abundance of prey species. Given that Bridle Shiner feeds on microcrustaceans, aquatic insects, detritus, and plants (COSEWIC 2013c), negative indirect effects are possible. Further studies are required to examine any potential indirect effects of Bayluscide on Bridle Shiner.

### **Relative Risk**

This study found that gB applications in the Great Lakes have overlapped with Bridle Shiner locations in the past (Table 2; Figure A5.37). For example, 1% of all ALIS segments within the range of Bridle Shiner have overlapped with a gB application location from 2011 to 2017. This represents less than 1% of all gB applications. This contributed to low spatial ( $R = 0.014$ ; 13<sup>th</sup> percentile) and intensity ( $I = 0.100$ ; 22<sup>nd</sup> percentile) values in comparison to other fishes.

Approximately 97% of all Bridle Shiner records were found within Types I and II habitat, resulting in a high habitat value in comparison to other fishes ( $H = 0.970$ ; 87<sup>th</sup> percentile). Given that toxicity information for this species is not available, the toxicity value in this study was based on the published  $LC_{50}$  for Fathead Minnow (mortality of 3.5% at a Bayluscide concentration of 0.057 mg/L over eight hours), resulting in the lowest toxicity value among fishes.

Overall risk for Bridle Shiner was low in comparison to other fish species (Figure 7;  $RR_M < 0.001$ ; 17<sup>th</sup> percentile), largely due to low exposure and toxicity of the surrogate species (Figure 6).

## **SILVER SHINER**

**Scientific Name:** *Notropis photogenis*

**Current COSEWIC Status and Year of Designation:** Threatened, May 2011

**SARA Schedule, Status and Year of Designation:** Schedule 1, Threatened, August 2019

**Current COSSARO Status and Year of Assessment:** Threatened, June 2011

### **Distribution**

In Canada, Silver Shiner is found in tributaries of Lake Ontario, Lake Erie and Lake St. Clair, including the Grand River, Thames River, Sixteen Mile Creek, Bronte Creek, and Saugeen River (COSEWIC 2011d, DFO unpublished data). Distribution in the Great Lakes for records since 1998 is provided in Figure A5.38.

### **Allowable Harm**

There are two competing hypotheses about Silver Shiner population dynamics. Under the short-lived hypothesis, population growth is most sensitive to perturbations that affect YOY survival, the fecundity of first time spawners, and the proportion of individuals that spawn at age 1 (Young and Koops 2013b). Such a population is largely insensitive to changes in survival or fertility of age 2 or 3 individuals. Under the long-lived hypothesis, population growth is most sensitive to changes in the survival of immature individuals (Young and Koops 2013b). For more information on Silver Shiner allowable harm, see Young and Koops (2013b).

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## Risk Associated with Granular Bayluscide

### Background

The tolerance of Silver Shiner to Bayluscide has not yet been examined but several studies have been conducted on a closely related species, Fathead Minnow. Marking and Hogan (1967) reported the species to experience mortality when exposed to Bayer 73 (LC<sub>50</sub> value of 0.106 ppm; pH of 7.5). Marking and Bills (1985) also reported a lethal concentration in Fathead Minnow (LC<sub>50</sub> of 0.11 mg/L) when exposed to Bayer 73, but found that when paired with 13 other contaminants including pesticides, heavy metals and industrial pollutants (DDT, Endrin, malathion, carbaryl, toxaphene, Delnav, cadmium copper, zinc, ammonia, nitrite, cyanide, and chlorine), toxicity was additive when exposed for 96 hours. Therefore, the potential for increased toxicity when combined with other chemicals, especially pollutants, should be considered when applying Bayluscide in areas with closely related species at risk, including Silver Shiner.

Silver Shiner feeds on aquatic insects, worms, crustaceans, water mites, and algae (COSEWIC 2011d). Aquatic insects such as caddisflies appear to be very susceptible to the effects of Bayluscide (Gilderhus 1979, Dawson 2003), which may result in a reduction in abundance and temporary shift of prey species. Further studies are required to examine any potential indirect effects of Bayluscide on Silver Shiner.

### Relative Risk

This study found that gB applications in the Great Lakes have had minimal overlap with Silver Shiner locations (Table 2, Figure A5.39). For example, only 2% of all ALIS segments within the range of Silver Shiner have overlapped with a gB application location from 2011 to 2017, which represents less than 1% of all gB applications and contributed to low spatial ( $R = 0.018$ ; 22<sup>nd</sup> percentile) and intensity values ( $I = 0.100$ ; 22<sup>nd</sup> percentile).

Approximately 22% of all Silver Shiner records were found within Types I and II habitat, resulting in a low habitat value in comparison to other fishes ( $H = 0.215$ ; 4<sup>th</sup> percentile). Given that toxicity information for this species is not available, the toxicity value in this study was based on the published LC<sub>50</sub> for Fathead Minnow (mortality of 3.5% at a Bayluscide concentration of 0.057 mg/L over eight hours), resulting in the lowest toxicity value for a fish species and tied with seven other species as a result of surrogate choice.

Overall risk to Silver Shiner was low ( $RR_M < 0.001$ ; 13<sup>th</sup> percentile; Figure 7) due to low exposure history, toxicity, and habitat values.

## NORTHERN MADTOM

**Scientific Name:** *Noturus stigmosus*

**Current COSEWIC Status and Year of Designation:** Endangered, May 2012

**SARA Schedule, Status and Year of Designation:** Schedule 1, Endangered, January 2005

**Current COSSARO Status and Year of Assessment:** Endangered, May 2012

### Distribution

In Canada, Northern Madtom is limited to four locations: Lake St. Clair, Detroit River, St. Clair River, and the Thames River. It is presumed extirpated from the Sydenham River (Holm and Mandrak 1998). Distribution in the Great Lakes for records since 1998 is provided in A5.40.

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## Risk Associated with Granular Bayluscide

### Background

A study by Boogaard et al. (2016b) using Tadpole Madtom (*Noturus gyrinus*), a surrogate of Northern Madtom, indicated that Tadpole Madtom display avoidance behaviour when exposed to granular 3.2% Bayluscide. The mortality of Tadpole Madtom in the columns treated with gB was high (67%), due to the species being confined and unable to swim away from the chemical, suggesting significant mortality may occur in the field if the application area is too large for escapement (Boogaard et al. 2016b). In addition, a study by Marking and Hogan (1967) showed that Bayer 73 is toxic to closely related *Ameiurus* spp., with 50% mortality ( $LC_{50}$ ) observed in Brown Bullhead and Black Bullhead (*Ameiurus melas*) exposed to concentrations of 0.071 ppm and 0.104 ppm (pH 7.5), respectively. Mortality of *Ameiurus* spp. has also been observed in the field after the application of Bayluscide. The SLCP indicated a total of 209 non-target mortalities of *Ameiurus* spp. after Bayluscide treatment from 1998 to 2014 (M. Steeves, SLCC, unpublished data).

Much of the diet of Northern Madtom consists of aquatic macroinvertebrates, including mayflies, caddisflies, and chironomids (COSEWIC 2012c). Mayflies appear to be relatively resistant to Bayluscide exposure (Gilderhus 1979, Bills et al. 1985). However, caddisflies and chironomids tend to be more susceptible (Gilderhus 1979). Gilderhus (1979) reported a 54% decline in population of chironomids seven days after treatment, and a complete elimination of caddisfly population 13 days after treatment with Bayer 73 (5% granular formulation). Therefore, the potential exists for local indirect effects on Northern Madtom caused by changes in food web structure following Bayluscide application. Further studies are required to examine potential indirect effects of Bayluscide on Northern Madtom.

### Relative Risk

This study found that gB applications in the Great Lakes have overlapped with Northern Madtom locations in the past (Figure A5.41). For example, approximately 22% of all ALIS segments within the range of Northern Madtom have overlapped with gB application locations from 2011 to 2017, which represents 12% of all gB applications and contributed to higher spatial ( $R = 0.216$ ; 87<sup>th</sup> percentile) and intensity values ( $I = 0.538$ ; 96<sup>th</sup> percentile) in comparison to other fishes. A total of five gB applications occurred within what is presently Northern Madtom critical habitat from 2012-2013 (Table 3; Figures A5.42 and A5.43).

Approximately 48% of all Northern Madtom records are found within Types I and II habitat, which resulted in a low habitat value in comparison to other fishes ( $H = 0.480$ ; 13<sup>th</sup> percentile).

Owing to the lack of specific toxicity information for Northern Madtom, Channel Catfish was used as a surrogate in the relative risk assessment. Based on the published  $LC_{50}$  for Channel Catfish, mortality of 53% at a Bayluscide concentration of 0.057 mg/L over eight hours was used in the risk assessment, placing the toxicity value for Northern Madtom in the top quartile among fishes ( $T = 0.532$ ).

Overall risk to Northern Madtom was very high in comparison to other fish species of conservation concern ( $RR_M = 0.0298$ ; 87<sup>th</sup> percentile; Figure 7), driven by high spatial, intensity, and toxicity values (Figure 6).

## PUGNOSE MINNOW

**Scientific Name:** *Opsopoeodus emiliae*

**Current COSEWIC Status and Year of Designation:** Threatened, May 2012

**SARA Schedule, Status and Year of Designation:** Schedule 1, Threatened, August 2019

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**Current COSSARO Status and Year of Assessment:** Threatened, May 2012

## **Distribution**

In Canada, Pugnose Minnow is limited to southwestern Ontario in the Detroit River and its tributary (Canard River), as well as Lake St. Clair and its tributaries (Sydenham River, Bear Creek, East Otter Creek, Chenail Ecarte, Little Bear Creek, Maxwell Creek, and Whitebread Drain). It is presumed extirpated from the Thames River and McDougall Drain (COSEWIC 2012d). Distribution in the Great Lakes for records since 1998 is provided in Figure A5.44.

## **Allowable Harm**

Pugnose Minnow populations are most sensitive to perturbations that affect the survival of immature individuals or the fertility of first time spawners. When a population trajectory is stable, transient harm (allowable one time removal, performed no more frequently than every four years) should not exceed a 5.5% reduction in YOY abundance, 28.5% reduction in adult abundance or a 4.5% reduction in total abundance, to avoid jeopardizing the survival and future recovery of Canadian populations (Young and Koops 2012). For more info information on Pugnose Minnow allowable harm, see (Young and Koops 2012).

## **Risk Associated with Granular Bayluscide**

### **Background**

The tolerance of Pugnose Minnow to Bayluscide exposure has not yet been examined but several studies have been conducted on a closely related species, Fathead Minnow. Fathead Minnow exhibited an LC<sub>50</sub> value of 0.106 ppm at pH 7.5, when exposed to Bayer 73 (LC<sub>50</sub> value of 0.106 ppm; pH of 7.5; Marking and Hogan 1967), but the toxicity of Bayer 73 is additive when paired with other contaminants including pesticides, heavy metals and industrial pollutants (e.g., DDT, Endrin, malathion, carbaryl, toxaphene, Delnav, cadmium copper, zinc, ammonia, nitrite, cyanide, and chlorine; Marking and Bills 1985). Therefore, the potential for increased toxicity when combined with other chemicals, especially pollutants, should be considered when applying Bayluscide in areas with closely related species at risk, including Pugnose Minnow.

Pugnose Minnow feeds on chironomid larvae, filamentous algae, small crustaceans, larval fish, and fish eggs (COSEWIC 2012d). Preferred prey species susceptible to the effects of Bayluscide include chironomid larvae and microcrustaceans (Shiff and Garnett 1961, Gilderhus 1979). Declines in abundance could result in temporary shortages of preferred prey and a shift to other prey species. Further studies are required to examine any potential indirect effects of Bayluscide on Pugnose Minnow.

### **Relative Risk**

This study found that gB applications in the Great Lakes have overlapped with Pugnose Minnow locations in the past (Table 2, Figure A5.45). For example, approximately 14% of all ALIS segments within the range of Pugnose Minnow have overlapped with gB application locations from 2011 to 2017, which represents less than 1% of all applications (Table 2) but resulted in a high spatial value ( $R = 0.143$ ; 70<sup>th</sup> percentile) in relation to other fishes. The intensity of applications was moderate for Pugnose Minnow ( $I = 0.200$ ; 52<sup>nd</sup> percentile).

Approximately 84% of all Pugnose Minnow records are found within Types I and II habitat, resulting in a high habitat preference value for the species in comparison with other fishes ( $H = 0.838$ ; 74<sup>th</sup> percentile).

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Specific toxicity information for this species is not available. Therefore, the toxicity value in this study was based on the published LC<sub>50</sub> for Fathead Minnow (mortality of 3.5% at a Bayluscide concentration of 0.057 mg/L over eight hours), resulting in the lowest toxicity value for a fish species, tied with seven other species as a result of surrogate choice.

Overall risk to Pugnose Minnow was moderate ( $RR_M < 0.001$ ; 52<sup>nd</sup> percentile; Figure 7).

## CHANNEL DARTER

**Scientific Name:** *Percina copelandi*

**Current COSEWIC Status and Year of Designation:** Special Concern (St. Lawrence populations), November 2016; Endangered (Lake Ontario populations), November 2016; Endangered (Lake Erie populations), November 2016.

**SARA Schedule, Status and Year of Designation:** Schedule 1, Special Concern, St. Lawrence populations; Schedule 1, Endangered, Lake Erie DU; Schedule 1, Endangered, Lake Ontario DU; August 2019

**Current COSSARO Status and Year of Assessment:** Special Concern, May 2017

### Distribution

Channel Darter is found in Ontario and Quebec. In Ontario, it has been collected along the shores and tributaries of the Huron-Erie corridor (Lake St. Clair, St. Clair River, Detroit River), shores of Lake Erie, and tributaries of Lake Ontario (Trent River, Moira River, Salmon River, Skootamatta River, and Black River) (DFO 2013b). In Quebec, populations are located in the tributaries of the upper St. Lawrence River and the Ottawa River. Distribution in the Great Lakes for records since 1998 is provided in Figure A5.46.

### Allowable Harm

Channel Darter populations are most sensitive to perturbations that affect survival in the first three years of life, and the fecundity of first- and second-time spawners. From a precautionary perspective, a maximum allowable harm of 6% for one and two year old individuals, or 10% for three year old individuals has been suggested to avoid jeopardizing the survival and future recovery of Canadian populations. For more info information on Channel Darter allowable harm, see Venturelli et al. (2010a).

## Risk Associated with Granular Bayluscide

### Background

The tolerance of Channel Darter to Bayluscide has not been investigated, but non-target mortality was observed in closely related Logperch (*Percina caprodes*) after the application of Bayer 73 (5% granular) to Lake Champlain river deltas in 1991 and 1995 (Fisheries Technical Committee 1999). Moreover, the DFO SLCC reported a total of 14 Logperch mortalities between 1998 and 2012 (M. Steeves, SLCC, unpublished data). Reductions in abundance of preferred prey, such as chironomids and ostracods, have also been observed after the application of Bayluscide (Shiff and Garnett 1961, Gilderhus 1979), which may result in temporary shifts in prey species. Further studies are required to examine Channel Darter-specific tolerances to Bayluscide exposure.

### Relative Risk

This study found that gB applications in the Great Lakes have overlapped with Channel Darter locations in the past (Table 2, Figure A5.47). For example, approximately 15% of all ALIS segments within the range of Channel Darter have overlapped with a gB application location

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from 2011 to 2017, which represents 10% of all gB applications and led to a high spatial value in comparison to other fishes ( $R = 0.148$ ; 74<sup>th</sup> percentile) and the highest intensity value of any species in this study ( $I = 1.00$ ). Furthermore, 30 gB applications have occurred within what is presently Channel Darter critical habitat from 2012-2017 (Table 3, Figure A5.48).

Approximately 67% of all Channel Darter records have occurred within Types I and II habitat, resulting in a low habitat value (35<sup>th</sup> percentile among fishes).

Toxicity in this study was based on potential mortality using information from its closest known surrogate, the Yellow Perch. Based on the published  $LC_{50}$  for Yellow Perch, a mortality of 4.6% at a Bayluscide concentration of 0.057 mg/L over eight hours was used in the risk assessment, resulting in a low toxicity value for this species in comparison to other fishes ( $T = 0.046$ ; 35<sup>th</sup> percentile).

Overall risk to Channel Darter was high in comparison to other fish species ( $RR_M = 0.0046$ ; 74<sup>th</sup> percentile; Figure 7), driven by high spatial and intensity values (Figure 6).

## RIVER DARTER

**Scientific Name:** *Percina shumardi*

**Designatable Unit (DU):** Great Lakes - Upper St. Lawrence populations

**Current COSEWIC Status and Year of Designation:** Endangered, April 2016

**SARA Schedule, Status and Year of Designation:** No Schedule, No Status

**Current COSSARO Status and Year of Assessment:** Endangered, December 2016

### Distribution

In Canada, River Darter can be found in Saskatchewan, Manitoba, and Ontario. In Saskatchewan, one record exists for the Saskatchewan River (COSEWIC 2016b). In Manitoba and northwestern Ontario, it can be found in the Assiniboine, Nelson, English, Rainy, Red, and Winnipeg rivers (Pratt et al. 2015). In northern Ontario, it occurs in the Attawapiskat, Albany, Severn, and Winisk river watersheds that drain into Hudson Bay (Pratt et al. 2015). In southern Ontario, it is known only from the Lake St. Clair drainage, which includes the Sydenham River and Thames River. Distribution in the Great Lakes for records since 1998 is provided in Figure A5.49.

### Allowable Harm

River Darter populations are sensitive to perturbations affecting young-of-year (YOY) survival rates and fertility (van der Lee and Koops 2020b). Harm to these aspects of life history should be avoided. Decreases in YOY-survival or fertility greater than 31–34% may result in population decline, assuming a population growth rate of 1.32 (van der Lee and Koops 2020b). Similarly, population may decline if mortality exceeds 24.5% for all age-classes. For more info information on River Darter allowable harm, see van der Lee and Koops (2020b).

## Risk Associated with Granular Bayluscide

### Background

The tolerance of River Darter to Bayluscide has not been reported but non-target mortality has been observed in closely related Logperch after the application of Bayer 73 (5% granular) to Lake Champlain river deltas in 1991 and 1995 (Fisheries Technical Committee 1999). Moreover, DFO's SLCC reported a total of 14 Logperch mortalities between 1998 and 2012 (M. Steeves, SLCC, unpublished data). Indirect effects on prey items could have negative effects on River Darter through a reduction in food availability. In a study by Pratt et al. (2016)



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dominant prey items for River Darter in Manitoba and northwestern Ontario were Diptera, Trichoptera, Ephemeroptera and zooplankton. Reductions in abundance of preferred prey, such as chironomids, have been observed after the application of Bayluscide (Shiff and Garnett 1961, Gilderhus 1979), which may result in temporary shifts in prey species. Further studies are required to examine River Darter-specific tolerances to Bayluscide exposure.

### **Relative Risk**

This study found that gB applications in the Great Lakes have overlapped with River Darter locations in the past (Table 2, Figure A5.50). For example, approximately 22% of all ALIS segments within the range of River Darter have overlapped with a gB application location, which represents less than 1% of all applications. This contributed to a high spatial ( $R = 0.222$ ; 91<sup>st</sup> percentile among fishes) and low intensity values ( $I = 0.150$ ; 39<sup>th</sup> percentile among fishes).

Approximately 55% of all River Darter occurrences have been documented within Types I and II habitat, resulting in a low habitat preference value for the species in comparison to other fishes ( $H = 0.545$ ; 26<sup>th</sup> percentile).

Given the lack of information regarding the sensitivity to Bayluscide for this species, toxicity in this study was based on the published  $LC_{50}$  for Yellow Perch (mortality of 4.6% at a Bayluscide concentration of 0.057 mg/L over eight hours), resulting in a low toxicity value ( $T = 0.046$ ; 35<sup>th</sup> percentile).

Overall risk to River Darter was moderate in comparison to all other fish species ( $RR_M < 0.001$ ; 57<sup>th</sup> percentile; Figure 7), as the higher spatial value was partially offset by lower habitat use and toxicity values.

## **MUSSEL SPECIES ACCOUNTS**

### **NORTHERN RIFFLESHELL**

**Scientific Name:** *Epioblasma rangiana*

**Current COSEWIC Status and Year of Designation:** Endangered, April 2010

**SARA Schedule, Status and Year of Designation:** Schedule 1, Endangered, June 2003

**Current COSSARO Status and Year of Assessment:** Endangered, June 2010

### **Distribution**

Historically, Northern Riffleshell was found in Lake Erie, Lake St. Clair and the Detroit, Thames, Ausable, and Sydenham rivers, but is now restricted to the east branch of the Sydenham River and the Ausable River. A single live individual was found in the St. Clair River delta in 1999, but has not been collected in that area since (COSEWIC 2010a). Distribution in the Great Lakes for records since 1998 is provided in Figure A5.51.

### **Risk Associated with Granular Bayluscide**

#### **Background**

No studies have examined the tolerance of the Northern Riffleshell or any of its potential host fishes to Bayluscide exposure. Further research is required to investigate potential direct and indirect impacts of Bayluscide application on this species. Given that toxicity information for this species is not available, the toxicity value in this study was based on potential mortality using information from its closest known surrogate, the Kidneyshell. Based on Newton et al.'s (2017) published  $LC_{50}$  for sub-adult Kidneyshell, a mortality of 54% at a Bayluscide concentration of 9.3 mg/L over eight hours was used in the risk assessment.

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## Relative Risk

This study found that gB applications in the Great Lakes have overlapped with Northern Riffleshell locations in the past (Figure A5.51). For example, approximately 3% of all ALIS segments within the range of Northern Riffleshell have overlapped with a gB application location from 2011 to 2017 (Table 2), which represents less than 1% of all applications. This was a low level of spatial overlap in comparison to all other mussel species ( $R = 0.031$ ; 29<sup>th</sup> percentile). Measuring the intensity of these applications where they have occurred revealed that the species scored the median value in this risk assessment category in relation to other mussels ( $I = 0.200$ ; 50<sup>th</sup> percentile).

Through the analysis of substrate records, it was found that approximately 81% of all Northern Riffleshell occurrences were found within Types I and II habitat. This resulted in a moderately high habitat preference value for the species in comparison to other mussels ( $H = 0.814$ ; 64<sup>th</sup> percentile). Furthermore, it was found that one gB application occurred within what is presently Northern Riffleshell critical habitat in 2012 (Table 3, Figure A5.52). The toxicity component of the relative risk equation was scored using Kidneyshell as a surrogate species. This resulted in a high toxicity value for Northern Riffleshell in relation to other mussels ( $T = 0.543$ ; 50<sup>th</sup> percentile).

The risk assessment found the overall score for Northern Riffleshell to be moderate in comparison to other mussel species ( $RR_M = 0.003$ ; Figure 9). The overall risk value placed Northern Riffleshell in the 43<sup>rd</sup> percentile for mussels. This is partly due to its relatively high habitat preference value being offset by its moderately low level of exposure to past gB applications (Figure 8).

## SNUFFBOX

**Scientific Name:** *Epioblasma triquetra*

**Current COSEWIC Status and Year of Designation:** Endangered, November 2011

**SARA Schedule, Status and Year of Designation:** Schedule 1, Endangered, June 2003

**Current COSSARO Status and Year of Assessment:** Endangered, November 2011

## Distribution

Historically, Snuffbox occurred in lakes Erie and St. Clair and the Ausable, Grand, Niagara, Sydenham, Detroit, and Thames rivers. Currently, it is restricted to the several sites in the Sydenham (east branch) and the Ausable rivers (COSEWIC 2011c). Distribution in the Great Lakes for records since 1998 is provided in Figure A5.53.

## Risk Associated with Granular Bayluscide

### Background

No studies have examined the tolerance of the Snuffbox or any of its potential host fishes to Bayluscide exposure. Further research is required to investigate potential direct and indirect impacts of Bayluscide application within the range of this species. Given that toxicity information for this species is not available, the toxicity value in this study was based on potential mortality using information from its closest known surrogate, the Kidneyshell. Based on Newton et al.'s (2017) published  $LC_{50}$  for sub-adult Kidneyshell, a mortality of 54% at a Bayluscide concentration of 9.3 mg/L over eight hours was used in the risk assessment.

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## Relative Risk

This study found that gB applications in the Great Lakes have overlapped with Snuffbox locations in the past (Figure A5.53). For example, approximately 3% of all ALIS segments within the range of Snuffbox have overlapped with a gB application location (Table 2), which represents less than 1% of all applications. This was a low level of spatial overlap in comparison to all other mussel species in this study ( $R = 0.033$ ; 36<sup>th</sup> percentile). Measuring the intensity of these applications where they have occurred, revealed that the species scored the median value in this risk assessment category in relation to other mussels in this study ( $I = 0.200$ ; 50<sup>th</sup> percentile).

Through the analysis of substrate records, approximately 66% of all Snuffbox occurrences were found within Types I and II habitat. This resulted in the lowest habitat preference value for any mussel species ( $H = 0.659$ ). Furthermore, one gB application occurred within what is presently Snuffbox critical habitat in 2012 (Table 3, Figure A5.54). The toxicity component of the relative risk equation was scored using Kidneyshell as a surrogate species, which resulted in a high toxicity value for Snuffbox in relation to other mussels ( $T = 0.543$ ; 50<sup>th</sup> percentile).

The risk assessment of gB use in the Great Lakes region found the overall value for Snuffbox to be moderately low in comparison to other mussel species ( $RR_M = 0.002$ ; Figure 9). The overall risk value placed Snuffbox in the 36<sup>th</sup> percentile for mussels, which was partly due to its high toxicity value being offset by low spatial and habitat use values.

## WAVYRAYED LAMPMUSSEL

**Scientific Name:** *Lampsilis fasciola*

**Current COSEWIC Status and Year of Designation:** Special Concern, April 2010

**SARA Schedule, Status and Year of Designation:** Schedule 1, Special Concern, March 2013

**Current COSSARO Status and Year of Assessment:** Threatened, June 2010

### Distribution

In Canada, Wavyrayed Lampmussel is currently restricted to the St. Clair River delta, upper Grand, Maitland, Thames, Sydenham and Ausable rivers. It appears to have been extirpated from western Lake Erie, Detroit River, and Lake St. Clair (excluding the St. Clair River delta) (DFO 2010). Distribution in the Great Lakes for records since 1998 is provided in Figure A5.55.

### Allowable Harm

Wavyrayed Lampmussel populations are most sensitive to perturbations of annual adult survival, and survival of glochidia and juveniles in the first year. Maximum allowable harm to annual survival of glochidia, juveniles, adults and fecundity should be limited to 14%, 9%, 6%, and 14%, respectively, to avoid jeopardizing the survival and future recovery of Canadian populations (Young and Koops 2010b). For more info information on Wavyrayed Lampmussel allowable harm, see Young and Koops (2010b).

## Risk Associated with Granular Bayluscide

### Background

Newton et al. (2017) found that exposure to gB at concentrations applied in the field (9.3 mg/L), had significant duration effects on mortality in sub-adult Wavyrayed Lampmussel. Mortality was observed in 51% of the population after 70 minutes of exposure ( $LT_{50}$ ) and sub-lethal responses

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(defined as gaped valves, production of mucus, and/or foot extension outside the shell) were observed after 107 min (ET<sub>50</sub>).

Of the known host fish species to the Wavyrayed Lampmussel, toxicity to Bayluscide has been reported in Largemouth Bass (*M. salmoides*) and Smallmouth Bass. Marking and Hogan (1967) reported an LC<sub>50</sub> value of 0.062 ppm for Largemouth Bass and LC<sub>50</sub> of 0.060 ppm for Smallmouth Bass after 96 hours of exposure to Bayer 73. A total of 18 Largemouth Bass and 24 Smallmouth Bass were also found dead after the application of Bayer 73 (5% granular) to five river deltas in 1991 and 1995 (Fisheries Technical Committee 1999). Therefore, a reduction in abundance of host fish species may occur after the application of Bayluscide.

### **Relative Risk**

This study found that gB applications in the Great Lakes have overlapped with Wavyrayed Lampmussel locations in the past (Table 2, Figure A5.55). For example, approximately 2% of all ALIS segments within the range of Wavyrayed Lampmussel have overlapped with a gB application location from 2011 to 2017 (Table 2), which represents less than 1% of all applications. This was a low level of spatial overlap in comparison to all other mussel species ( $R = 0.016$ ; 14<sup>th</sup> percentile). Measuring the intensity of these applications where they have occurred, revealed that the species scored a low value in this risk assessment category in relation to other mussels ( $I = 0.133$ ; 21<sup>st</sup> percentile).

The analysis of substrate records indicated that approximately 77% of all Wavyrayed Lampmussel occurrences were from Types I and II habitat. This resulted in a moderate habitat preference value for the species in comparison to other mussels ( $H = 0.768$ ; 43<sup>rd</sup> percentile). A mortality of 51%, based on Newton et al. (2017), was used as the toxicity value, which resulted in a moderate toxicity value for Wavyrayed Lampmussel in relation to other mussel species ( $T = 0.508$ ; 43<sup>rd</sup> percentile).

The risk assessment found the overall relative risk value for Wavyrayed Lampmussel to be low in comparison to other mussel species ( $RR_M = 0.001$ ; Figure 9). The overall risk value placed Wavyrayed Lampmussel in the 21<sup>st</sup> percentile for mussels, which was largely due to its low exposure history to gB application as indicated through geospatial analysis (Figure 8).

## **EASTERN PONDMUSSEL**

**Scientific Name:** *Ligumia nasuta*

**Current COSEWIC Status and Year of Designation:** Special Concern, April 2017

**SARA Schedule, Status and Year of Designation:** Schedule 1, Special Concern, August 2019

**Current COSSARO Status and Year of Assessment:** Special Concern, November 2017

### **Distribution**

Eastern Pondmussel has been known from Lake Ontario, Lake Erie, Lake St. Clair and the Detroit River. However, it is believed to have been lost from approximately 93% of its former range in Canada due to the impact of Zebra Mussel (*Dreissena polymorpha*) (COSEWIC 2007a). Its current distribution includes lakes Erie, Ontario, and St. Clair and their various connecting channels and coastal wetland areas. Eastern Pondmussel has been observed in a number of inland lakes in eastern Ontario (DFO unpublished data). Distribution in the Great Lakes for records since 1998 is provided in Figure A5.56.

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## Allowable Harm

Allowable harm for Eastern Pondmussel has not been estimated because of a lack of data on population growth rates and projections. However, Young and Koops (2011a) predicted which vital rates were likely to be most sensitive to harm. They found that Eastern Pondmussel population growth is most sensitive to adult survival and somewhat sensitive to juvenile survival. Therefore, harm to these life-history stages should be minimized to avoid jeopardizing the survival and future recovery of Canadian populations. For more info information on Eastern Pondmussel allowable harm, see Young and Koops (2011a).

## Risk Associated with Granular Bayluscide

### Background

A study by Newton et al. (2017) found that exposure to granular 3.2% Bayluscide at concentrations applied in the field (9.3 mg/L) had significant duration effects on mortality and sub-lethal responses (defined as gaped valves, production of mucus, and/or foot extension outside the shell) in adult Eastern Pondmussel. Of the 58 mussels exposed, mortality was observed in 15.5% of population, and sub-lethal responses were observed in 50% of the population after 320 minutes of exposure (Newton et al. 2017). However, the majority of mussels (60%) were able to recover after 21 days despite these displays (Newton et al. 2017). The mortality estimate from Newton et al. (2017) was used to inform the toxicity value in this study's risk assessment.

Of the known host fishes for the Eastern Pondmussel, studies have examined the effects of Bayluscide on Yellow Perch. This species appears to experience mortality to Bayer 73, having an  $LC_{50}$  of 0.082 ppm after 24 h of exposure (pH of 7.5; Marking and Hogan 1967). The SLCP also reported the mortality of 12 Yellow Perch after the application of Bayluscide from 1998 to 2013 (M. Steeves, SLCC, unpublished data). A reduction in host fish species may have adverse effects on the Canadian population of Eastern Pondmussel.

### Relative Risk

This study found that gB applications in the Great Lakes have overlapped with Eastern Pondmussel locations in the past (Table 2, Figure A5.57). For example, approximately 5% of all ALIS segments within the range of Eastern Pondmussel have overlapped with a gB application location from 2011 to 2017 (Table 2), which represents less than 2% of all applications. This was a near median level of spatial overlap in comparison to all other mussel species in this study ( $R = 0.045$ ; 57<sup>th</sup> percentile). Measuring the intensity of these applications where they have occurred, indicated a high intensity value in this risk assessment category in relation to other mussels ( $I = 0.425$ ; 93<sup>rd</sup> percentile)

Based on published information of habitat use of Eastern Pondmussel, the species prefers fine sand and muddy substrates (COSEWIC 2017). Furthermore, four gB applications occurred within what was previously identified as Eastern Pondmussel critical habitat (proposed) in 2014 (Table 3, Figure A5.58). An analysis of substrate records found that 100% of all Eastern Pondmussel occurrences were found within Types I and II habitat. This resulted in the highest habitat preference value for all mussel species ( $H = 1$ ; tied with Lilliput). To score toxicity to gB, a mortality of 16% based on Newton et al. (2017) was used for this species. This resulted in a low toxicity value for Eastern Pondmussel in relation to all other mussel species ( $T = 0.155$ ; 14<sup>th</sup> percentile).

The risk assessment of gB use in the Great Lakes region found the overall value for Eastern Pondmussel to be moderate in comparison to other mussel species ( $RR_M = 0.003$ ; Figure 9).

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The overall risk value placed Eastern Pondmussel in the 50<sup>th</sup> percentile for mussels, which was partly due to a relatively low toxicity value in comparison to other mussel species (Figure 8).

## **THREEHORN WARTYBACK**

**Scientific Name:** *Obliquaria reflexa*

**Current COSEWIC Status and Year of Designation:** Threatened, May 2013

**SARA Schedule, Status and Year of Designation:** Schedule 1, Threatened, August 2019

**Current COSSARO Status and Year of Assessment:** Threatened, June 2013

### **Distribution**

In Canada, Threehorn Wartyback was historically known from Lake St. Clair, western Lake Erie and the Grand, Thames, Sydenham, and Detroit rivers (COSEWIC 2013d). Currently, it is restricted to the Sydenham, Grand, and Thames rivers (DFO 2014a). Distribution in the Great Lakes for records since 1998 is provided in Figure A5.59.

### **Allowable Harm**

Allowable harm has not been estimated because of insufficient information on life history of Threehorn Wartyback. However, using an updated version of the classification model by Young and Koops (2011a), Threehorn Wartyback appears to fall into a “low sensitivity” group, where population growth is equally sensitive to changes in adult survival, juvenile survival, and lifespan (DFO 2014a). Therefore, harm to these life-history stages should be minimized to avoid jeopardizing the survival and future recovery of Canadian populations. For more info information on Threehorn Wartyback allowable harm, see DFO (2014a).

### **Risk Associated with Granular Bayluscide**

#### **Background**

There have been very few studies on the toxicity of Bayluscide to Threehorn Wartyback. Waller et al. (1993) exposed the Threehorn Wartyback (30 to 50 mm in size) to Bayluscide for eight hours at 17°C and reported the LC<sub>50</sub> as 0.051 mg/L. Delayed mortality was also observed eight hours post-exposure and resulted in a lower LC<sub>50</sub> value of 0.0445 mg/L. Given a lack of mortality information for Bayluscide exposure over eight hours at 9.3 mg/L, the toxicity value for this species was based on mortality in a surrogate, the Kidneyshell, published by Newton et al. (2017).

Indirect effects on this mussel species include impacts to host fishes. Toxicity tests have not been conducted on its host fish species but mortality of the Longnose Dace (*Rhinichthys cataractae*) (~ 81 individuals) was observed after the application of Bayer 73 (5% granular) to the Boquet and Salmon River in Lake Champlain (Fisheries Technical Committee 1999).

#### **Relative Risk**

This study found that gB applications in the Great Lakes have overlapped with Threehorn Wartyback locations in the past (Table 2, Figure A5.59). For example, approximately 13% of all ALIS segments within the range of Threehorn Wartyback have overlapped with a gB application location from 2011 to 2017 (Table 2), which represents less than 1% of all applications. This was a high spatial value in comparison to all other mussel species ( $R = 0.130$ ; 79<sup>th</sup> percentile). Measuring the intensity of these applications where they have occurred, revealed that the species scored a low value in this risk assessment category in relation to other mussels in this study ( $I = 0.133$ ; 21<sup>st</sup> percentile).

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Through the analysis of substrate records, it was found that approximately 76% of all Threehorn Wartyback were found within Types I and II habitat. This resulted in a moderately low habitat preference value for the species in comparison to other mussels ( $H = 0.763$ ; 36<sup>th</sup> percentile). The toxicity component of the relative risk equation was scored using Kidneyshell as a surrogate species, which resulted in a high toxicity value for Threehorn Wartyback in relation to other mussels ( $T = 0.543$ ; 50<sup>th</sup> percentile).

The risk assessment found the overall relative risk value for Threehorn Wartyback to be high in comparison to other mussel species ( $RR_M = 0.007$ ; Figure 9). The overall risk value placed Threehorn Wartyback in the 93<sup>rd</sup> percentile for mussels, which was largely due to relatively high exposure to past gB applications (Figure 8).

## HICKORYNUT

**Scientific Name:** *Obovaria olivaria*

**Current COSEWIC Status and Year of Designation:** Endangered, May 2011

**SARA Schedule, Status and Year of Designation:** Schedule 1, Endangered, August 2019

**Current COSSARO Status and Year of Assessment:** Endangered, June 2011

### Distribution

In Canada, Hickorynut inhabits the Great Lakes and St. Lawrence River watershed. Currently, it is extant in the Mississagi River (Lake Huron), the Ottawa River and its tributaries (Blanche and Coulonge rivers), and the St. Lawrence River and its tributaries (Batiscan River, Rivière St. François, Rivière L'Assomption) (DFO 2013a). It is presumed extirpated from the Detroit River and Niagara River due to loss of host fish and presence of dreissenid mussels (COSEWIC 2011a). Distribution in the Great Lakes for records since 1998 is provided in Figure A5.60.

### Allowable Harm

Hickorynut population growth is most sensitive to perturbations that affect juvenile or adult survival. In addition, if host fish (Lake Sturgeon) abundance is limiting, Hickorynut viability becomes sensitive to the rate of glochidial attachment (Young and Koops 2013c). Therefore, harm to these life-history stages or harm that restricts host availability for glochidial attachment should be minimized to avoid jeopardizing the survival and future recovery of Canadian populations. For more info information on Hickorynut allowable harm, see Young and Koops (2013c).

### Risk Associated with Granular Bayluscide

#### Background

A study by Newton et al. (2017) found that exposure to granular 3.2% Bayluscide at concentrations applied in the field (9.3 mg/L), had significant duration effects on mortality and sub-lethal responses (defined as gaped valves, production of mucus, and/or foot extension outside the shell) in sub-adult Hickorynut. Nine hours of exposure resulted in a mortality rate of approximately 23% for both adults and sub-adult Hickorynut. Despite similar mortality rates, mortality responses differed between life stages. In sub-adults, mortality began after 45 minutes, whereas in adults, mortality was not observed until 360 minutes. Sub-lethal responses were also much more delayed in adults ( $ET_{50}$  of 423 min. vs. 153 min.) and recovery was less likely.

Indirect effects of Bayluscide exposure include impacts to host fishes. The host fish for Hickorynut in Canada is believed to be the Lake Sturgeon (COSEWIC 2011a). Although studies have not investigated the lethal concentration limits of this species, it appears as though it has

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the ability to detect and avoid gB applications (Boogaard et al. 2008b). Movement of this host species away from areas where Hickorynut is known to occur may result in a reduction in Hickorynut recruitment and possible recruitment failure.

### **Relative Risk**

This study found that gB applications in the Great Lakes have overlapped with Hickorynut locations in the past (Table 2, Figure A5.61). For example, approximately 17% of all ALIS segments within the range of Hickorynut have overlapped with a gB application location from 2011 to 2017 (Table 2), which represents less than 1% of all applications. This was the highest spatial value (tied with Salamander Mussel) in comparison to all other mussel species ( $R = 0.167$ ). This high spatial value reflects the limited distribution of this species in Ontario but it also may be inflated due to incomplete distribution data for the Ottawa River. Measuring the intensity of these applications where they have occurred within its range, revealed that the species scored the median value in this risk assessment category in relation to other mussels in this study ( $I = 0.200$ ; 50<sup>th</sup> percentile).

Based on published information on habitat use for Hickorynut, the species uses sandy or silty sand substrates (COSEWIC 2011a). Through the analysis of substrate records, approximately 84% of all Hickorynut were found within Types I and II habitat. This resulted in a high habitat preference value for the species in comparison to other mussels ( $H = 0.840$ ; 79<sup>th</sup> percentile). To score toxicity to gB, a mortality of 23%, based on Newton et al. (2017), was used for this species, which resulted in a low toxicity value for Hickorynut in relation to all other mussel species ( $T = 0.233$ ; 29<sup>th</sup> percentile).

The risk assessment of gB use in the Great Lakes region found the overall relative risk value for Hickorynut to be high in comparison to all other mussel species ( $RR_M = 0.007$ ; 86<sup>th</sup> percentile; Figure 9). The high score was largely due to the high exposure to past gB applications.

## **ROUND HICKORYNUT**

**Scientific Name:** *Obovaria subrotunda*

**Current COSEWIC Status and Year of Designation:** Endangered, May 2013

**SARA Schedule, Status and Year of Designation:** Schedule 1, Endangered, January 2005

**Current COSSARO Status and Year of Assessment:** Endangered, June 2013

### **Distribution**

In Canada, Round Hickorynut was historically distributed in Lake Erie, Lake St. Clair and the Welland, Grand, Thames, Sydenham, and Detroit rivers (COSEWIC 2003). It is now limited to the St. Clair River delta and the east branch of the Sydenham River (DFO 2013c). Distribution in the Great Lakes for records since 1998 is provided in Figure A5.62.

## **Risk Associated with Granular Bayluscide**

### **Background**

A mortality rate of 44% in adult Round Hickorynut exposed to granular 3.2% Bayluscide at concentrations applied in the field (9.3 mg/L) was observed by Newton et al. (2017). Mortality was observed in 50% of the population after 105 minutes of exposure ( $LT_{50}$ ) and sub-lethal responses (defined as gaped valves, production of mucus, and/or foot extension outside the shell) were observed after 279 minutes ( $ET_{50}$ ). Among those that displayed sub-lethal responses, none were able to recover. This information was used to score toxicity to Bayluscide for this species in our risk assessment.



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## Relative Risk

This study found that gB applications in the Great Lakes have overlapped with Round Hickorynut locations in the past (Table 2, Figure A5.62). For example, approximately 8% of all ALIS segments within the range of Round Hickorynut have overlapped with a gB application location from 2011 to 2017 (Table 2), which represents less than 1% of all applications. This was a moderately high spatial value in comparison to all other mussel species ( $R = 0.077$ ; 71<sup>st</sup> percentile). The intensity of these applications were moderate in relation to other mussels in this study ( $I = 0.200$ ; 50<sup>th</sup> percentile).

An analysis of substrate records found that 67% of all Round Hickorynut were found within Types I and II habitat, which resulted in a very low habitat preference value for all mussel species ( $H = 0.667$ ; 7<sup>th</sup> percentile). Furthermore, it was found that 4 gB applications occurred within what is presently Round Hickorynut critical habitat in 2012 (Table 3, Figure A5.63). To score toxicity to gB, a mortality of 44%, based on Newton et al. (2017), was used for this species. This resulted in a low toxicity value for Round Hickorynut in relation to all other mussel species ( $T = 0.444$ ; 36<sup>th</sup> percentile).

The risk assessment found the overall relative risk value for Round Hickorynut to be moderately high in comparison to all other mussel species ( $RR_M = 0.005$ ; Figure 9). The overall risk value placed Round Hickorynut in the 64<sup>th</sup> percentile for mussels, which was partly due to its high exposure to past gB applications (Figure 8).

## ROUND PIGTOE

**Scientific Name:** *Pleurobema sintoxia*

**Current COSEWIC Status and Year of Designation:** Endangered, May 2004

**SARA Schedule, Status and Year of Designation:** Schedule 1, Endangered, July 2005

**Current COSSARO Status and Year of Assessment:** Endangered, December 2014

## Distribution

Round Pigtoe was historically collected from the Niagara, Detroit, Grand, Thames, and Sydenham rivers, as well as Lake Erie and Lake St. Clair. Its current distribution is restricted to Lake Erie in Rondeau Bay, St. Clair River delta, the Grand, Thames, and Sydenham rivers (DFO 2018, DFO unpublished data). Distribution in the Great Lakes for records since 1998 is provided in Figure A5.64.

## Risk Associated with Granular Bayluscide

### Background

A study by Newton et al. (2017) reported that exposure to granular 3.2% Bayluscide at concentrations applied in the field (9.3 mg/L), resulted in a mortality rate of 22.4% in adult Round Pigtoe after eight hours of exposure. The study also found statistically significant duration effects on sub-lethal responses (defined as gaped valves, production of mucus, and/or foot extension outside the shell) in adult Round Pigtoe. The median exposure duration of Bayluscide needed to observe a sub-lethal response in 25% ( $ET_{25}$ ) of Round Pigtoe was 314 minutes. Of the 60 individuals exposed, 5 displayed sub-lethal responses and 3 of these recovered after 21 days. The mortality reported in this study was used to score toxicity to Bayluscide in this risk assessment.

Of the known host fish species, toxicity to Bayluscide has been reported in the Bluegill. Marking and Hogan (1967) reported the species to experience mortality when exposed to Bayer 73 (LC<sub>50</sub>

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value of 0.094 ppm after 96 hours at 12°C), but toxicity increased at higher temperatures (LC<sub>50</sub> value of 0.068 at 17°C). Laboratory experiments have also examined the effects of the 70% wettable powder formulation of Bayer 73, and found Bluegill to be relatively resistant with an LC<sub>50</sub> value of 0.152 mg/L (Bills and Marking 1976). Mortality of Bluegill has also been observed in the field after the application of the 5% granular formulation (Fisheries Technical Committee 1999). A reduction in host fishes may negatively affect the recruitment potential of Canadian populations of Round Pigtoe.

### Relative Risk

This study found that gB applications in the Great Lakes have overlapped with Round Pigtoe locations in the past (Table 2, Figure A5.64). For example, approximately 2% of all ALIS segments within the range of Round Pigtoe have overlapped with a gB application location from 2011 to 2017 (Table 2), which represents less than 1% of all applications. This was a low level of spatial overlap in comparison to all other mussel species ( $R = 0.016$ ; 7<sup>th</sup> percentile). The intensity of these applications where they have occurred within its range, were low in relation to other mussels in this study. This resulted in the lowest value for all mussel species in this scoring category ( $I = 0.050$ ; tied with Kidneyshell).

An analysis of substrate records indicated that approximately 75% of all Round Pigtoe were found within Types I and II habitat, which resulted in a low habitat preference value for the species in comparison to other mussels ( $H = 0.748$ ; 29<sup>th</sup> percentile). Furthermore, it was found that one gB application occurred within what is presently Round Pigtoe critical habitat in 2012 (Table 3; Figure A5.65). To score toxicity to gB, a mortality of 22%, based on Newton et al. (2017), was used for this species. This resulted in a low toxicity value for Round Pigtoe in relation to all other mussel species ( $T = 0.224$ ; 21<sup>st</sup> percentile).

The risk assessment found the overall relative risk value for Round Pigtoe to be the second lowest in comparison to all other mussel species ( $RR_M < 0.001$ ; 7<sup>th</sup> percentile; Figure 9), which was due to low values in all components of the risk assessment.

## KIDNEYSHELL

**Scientific Name:** *Ptychobranchus fasciolaris*

**Current COSEWIC Status and Year of Designation:** Endangered, May 2013

**SARA Schedule, Status and Year of Designation:** Schedule 1, Endangered, January 2005

**Current COSSARO Status and Year of Assessment:** Endangered, June 2013

### Distribution

The historical distribution of Kidneyshell in Canada was Lake St.Clair, Lake Erie, as well as the Ausable, Detroit, Grand, Niagara, Sydenham, Thames, and Welland rivers. It is now restricted to the Ausable, Sydenham, and Thames (Medway Creek) rivers and the St. Clair River delta (DFO 2013c). Distribution in the Great Lakes for records since 1998 is provided in Figure A5.66.

## Risk Associated with Granular Bayluscide

### Background

Kidneyshell is particularly sensitive to exposure of granular 3.2% Bayluscide at concentrations applied in the field (9.3 mg/L; Newton et al. 2017). Of the eight mussel species examined, mortality rate was the highest in sub-adult Kidneyshell (54%). Mortality was observed in 50% of the population after 53 minutes of exposure (LT<sub>50</sub>) and sub-lethal responses (defined as gaped valves, production of mucus, and/or foot extension outside the shell) were observed after 252 minutes (ET<sub>50</sub>). Among those that displayed a response, recovery was observed in only 25% of

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the population. No information exists on the tolerances of host fishes to exposure to Bayluscide. The sub-adult mortality given in Newton et al. (2017) was used to score toxicity in the relative risk assessment for Kidneyshell.

### **Relative Risk**

This study found that gB applications in the Great Lakes have overlapped with Kidneyshell locations in the past (Table 2, Figure A5.66). For example, approximately 2% of all ALIS segments within the range of Kidneyshell have overlapped with a gB application location from 2011 to 2017 (Table 2), which represents less than 1% of all applications. This was a low level of spatial overlap in comparison to all other mussel species ( $R = 0.023$ ; 21<sup>st</sup> percentile). The intensity of these applications were low in relation to other mussels in this study. This resulted in the lowest value for all mussel species in this category ( $I = 0.050$ ; tied with Round Pigtoe).

Through the analysis of substrate records, approximately 78% of all Kidneyshell were found within Types I and II habitat which resulted in a moderate habitat preference value for the species in comparison to other mussels ( $H = 0.776$ ; 50<sup>th</sup> percentile). Furthermore, five gB applications occurred within what is presently Kidneyshell critical habitat in 2012 (Table 3, Figure A5.67). To assign toxicity to gB, a mortality of 54% based on Newton et al. (2017) was used for this species. This resulted in a high toxicity value for Kidneyshell in relation to all other mussel species ( $T = 0.543$ ; 50<sup>th</sup> percentile).

The risk assessment found the overall relative risk value for Kidneyshell to be low in comparison to other mussel species ( $RR_M < 0.001$ ; Figure 9). The overall risk value placed Kidneyshell in the 14<sup>th</sup> percentile for mussels which was due to low values in the spatial and intensity components of the risk assessment.

### **MAPLELEAF**

**Scientific Name:** *Quadrula quadrula*

**Designatable Unit (DU):** Great Lakes - Upper St. Lawrence population

**Current COSEWIC Status and Year of Designation:** Special Concern, November 2016

**SARA Schedule, Status and Year of Designation:** Schedule 1, Special Concern, August 2019

**Current COSSARO Status and Year of Assessment:** Special Concern, May 2017

### **Distribution**

In Canada, Mapleleaf populations are separated into two Designatable Units (DUs): the Saskatchewan-Nelson population (Manitoba DU) and the Great Lakes-Upper St. Lawrence population (Ontario DU). In Manitoba, it has been collected from the Assiniboine, Berens, Bloodvein, Bradbury, Brokenhead, La Salle, Maskwa, Pigeon, Rat, Red, Roseau, and Wanipagow rivers as well as Cooks Creek. In Ontario, the distribution is restricted to the tributaries of Lake St. Clair (Sydenham and Thames rivers and some of their tributaries, Ruscom River), Lake Huron (Ausable and Bayfield rivers, Cow and Perch creeks), Lake Erie (Grand and Welland rivers and some of their tributaries and Lake Henry on Pelee Island) as well as the coastal wetland areas and tributaries in Lake Ontario (Cootes Paradise, Jordan Harbour, Fifteen and Sixteen Mile creeks) (DFO 2011, DFO unpublished data). A single individual has also been found in both the St. Clair River delta and Bayfield River (DFO 2011). Mapleleaf is believed to be extirpated from the Great Lakes proper and their connecting channels (DFO 2011). Distribution in the Great Lakes for records since 1998 is provided in Figure A5.68.

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## Allowable Harm

Allowable harm for Mapleleaf has not been estimated because of a lack of data on population growth rates and projections. However, Young and Koops (2011a) predicted which vital rates were likely to be most sensitive to harm. They found that Mapleleaf population growth is most sensitive to adult survival and somewhat sensitive to juvenile survival. Therefore, harm to these life-history stages should be minimized to avoid jeopardizing the survival and future recovery of Canadian populations. For more info information on Mapleleaf allowable harm, see Young and Koops (2011a).

## Risk Associated with Granular Bayluscide

### Background

A toxicity study by Newton et al. (2017) reported no significant effects on exposure duration to mortality or sub-lethal responses (defined as gaped valves, production of mucus, and/or foot extension outside the shell) in Mapleleaf. Mortality was observed in only 3% of the population and 100% recovery was observed in the few individuals who displayed sub-lethal responses. Therefore, the application of granular 3.2% Bayluscide at concentrations applied in the field (9.3 mg/L) appears to have little effect on Mapleleaf. The level of mortality observed in Newton et al. (2017) was used to evaluate toxicity for this species in the relative risk assessment.

Tolerance to Bayluscide exposure has also been examined in the host fish species, the Channel Catfish. Marking and Hogan (1967) reported that the fish experienced mortality to Bayluscide, having an  $LC_{50}$  of 0.082 ppm (pH of 7.5, 17°C) after 96 hours of exposure. However, Bills and Marking (1976) reported a lower  $LC_{50}$  value of 0.0370 mg/L (pH of 7.5, 12°C) after 96 hours of exposure to the 70% wettable powder formulation of Bayer 73.

### Relative Risk

This study found that gB applications in the Great Lakes have overlapped with Mapleleaf locations in the past (Table 2, Figure A5.68). For example, approximately 4% of all ALIS segments within the range of Mapleleaf have overlapped with a gB application location from 2011 to 2017 (Table 2), which represents less than 1% of all applications. This was a moderate level of spatial overlap in comparison to all other mussel species in this study ( $R = 0.036$ ; 43<sup>rd</sup> percentile). The intensity of these applications were low in relation to other mussels, resulting in a moderately low value ( $I = 0.138$ ; 36<sup>th</sup> percentile).

An analysis of substrate records indicated that approximately 78% of all Mapleleaf were found within Types I and II habitat. This resulted in a moderate habitat preference value for the species in comparison to other mussels ( $H = 0.777$ ; 57<sup>th</sup> percentile). Furthermore, it was found that 14 gB applications occurred within what was previously identified as Mapleleaf critical habitat (Proposed) from 2012-2014 (Table 3, Figures A5.69 and A5.70). To assign toxicity to gB, a mortality of 3% based on Newton et al. (2017) was used for this species. This resulted in the lowest toxicity value among all mussel species ( $T = 0.033$ ).

The risk assessment found the overall relative risk value for Mapleleaf to be the lowest in comparison to all other mussel species ( $RR_M < 0.001$ ; Figure 9), which was largely due to gB being less toxic to this species than other mussel species at risk.

## SALAMANDER MUSSEL

**Scientific Name:** *Simpsonaias ambigua*

**Current COSEWIC Status and Year of Designation:** Endangered, May 2011

**SARA Schedule, Status and Year of Designation:** Schedule 1, Endangered, June 2003

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**Current COSSARO Status and Year of Assessment:** Endangered, June 2011

## **Distribution**

In Canada, Salamander Mussel was historically known from the Detroit, Thames, and Sydenham rivers, but it is now restricted to the east branch of the Sydenham River DFO (2018). Distribution in the Great Lakes for records since 1998 is provided in Figure A5.71.

## **Risk Associated with Granular Bayluscide**

### **Background**

The toxicity of Bayluscide to Salamander Mussel and its host the Mudpuppy salamander is unknown. However, toxicity of TFM combined with Niclosamide (active ingredient in Bayluscide) on juvenile and adult mudpuppies have been examined. Adult mudpuppies appear to be resistant to TFM: 1% Niclosamide mixture at concentrations 1.5 times greater than the minimal lethal concentration required to kill Sea Lamprey (Boogaard et al. 2003), and juveniles appear to be at a greater risk of treatment related mortality at concentrations as low as 0.6 times the concentration to kill Sea Lamprey (Boogaard et al. 2008a). Given a lack of mortality information for Bayluscide exposure over eight hours at 9.3 mg/L, the toxicity for this species was based on its surrogate, the Kidneyshell. Therefore, a mortality of 54% was used in the risk assessment for Salamander Mussel.

### **Relative Risk**

This study found that gB applications in the Great Lakes have had high overlap with Salamander Mussel locations in the past (Table 2, Figure A5.71). For example, approximately 17% of all ALIS segments within the range of Salamander Mussel have overlapped with a gB application location from 2011 to 2017 (Table 2), which represents less than 1% of all applications. This was the highest level of spatial overlap with gB applications in comparison to all other mussel species (Tied with Hickorynut;  $R = 0.167$ ). The intensity of applications was moderate in relation to other mussels in this study. This resulted in Salamander Mussel scoring the median value for the intensity component of the risk assessment ( $I = 0.200$ ; 50<sup>th</sup> percentile).

An analysis of substrate records found that 71% of all Salamander Mussel were found within Types I and II habitat, which resulted in a low habitat preference value for the species in comparison to other mussels ( $H = 0.706$ ; 21<sup>st</sup> percentile). Furthermore, it was found that one gB application occurred within what is presently Salamander Mussel critical habitat in 2012 (Table 3, Figure A5.72). The toxicity component of the relative risk equation was evaluated using Kidneyshell as a surrogate species, which resulted in a high toxicity value for Salamander Mussel in relation to other mussels ( $T = 0.543$ ; 50<sup>th</sup> percentile).

The risk assessment found the overall relative risk value for Salamander Mussel to be the highest in comparison to all other mussel species ( $RR_M = 0.013$ ; Figure 9), which was largely due its very high spatial and toxicity values (Figure 8).

## **LILLIPUT**

**Scientific Name:** *Toxolasma parvum*

**Current COSEWIC Status and Year of Designation:** Endangered, May 2013

**SARA Schedule, Status and Year of Designation:** Schedule 1, Endangered, August 2019

**Current COSSARO Status and Year of Assessment:** Threatened, June 2013

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## Distribution

Historically, Lilliput was recorded from the Detroit, Sydenham, Thames, and Grand rivers. Its current distribution is limited to three tributaries of Lake St. Clair [Sydenham (east branch), Belle, and Ruscom rivers], Baptiste Creek (Thames River tributary), Grand River, Welland River, Oswego Creek, Jordan Harbour, Pelee Island, and Hamilton Harbour (Cootes Paradise, Carroll's Bay, Grindstone and Spencer creeks, Sunfish Pond) (DFO 2014c, DFO unpublished data). Distribution in the Great Lakes for records since 1998 is provided in Figure A5.73.

## Allowable Harm

Allowable harm has not been estimated because of insufficient information on the life history of Lilliput. However, using an updated version of the classification model by Young and Koops (2011a), Lilliput appears to fall into a "low sensitivity" group, where population growth is equally sensitive to changes in adult survival, juvenile survival and lifespan (DFO 2014c). Therefore, harm to these life-history stages should be minimized to avoid jeopardizing the survival and future recovery of Canadian populations. For more info information on Lilliput allowable harm, see DFO (2014c).

## Risk Associated with Granular Bayluscide

### Background

No studies have examined the tolerance of Lilliput to Bayluscide exposure. Although the direct effects of Bayluscide exposure on mortality have not be tested, toxicity tests have been conducted on one of its host fishes, the Bluegill. Impacts on host species are an important pathway in which indirect effects caused by Bayluscide could be observed in mussels. Marking and Hogan (1967) reported that the species experienced mortality when exposed to Bayer 73 (LC<sub>50</sub> value of 0.094 ppm after 96 hours at 12°C), but toxicity increased at higher temperatures (LC<sub>50</sub> value of 0.068 ppm at 17°C). Laboratory experiments have also examined the effects of the 70% wettable powder formulation of Bayer 73 and found Bluegill to be relatively resistant with an LC<sub>50</sub> value of 0.152 mg/L (Bills and Marking 1976). Mortality of Bluegill has also been observed in the field after the application of the 5% granular formulation (Fisheries Technical Committee 1999). A reduction in host fishes may negatively affect the recruitment potential of Canadian populations of Lilliput.

### Relative Risk

This study found that gB applications in the Great Lakes have overlapped with Lilliput locations in the past (Table 2, Figure A5.74). For example, approximately 7% of all ALIS segments within the range of Lilliput have overlapped with a gB application location from 2011 to 2017 (Table 2), which represents less than 1% of all applications. This was a moderately high spatial value in comparison to all other mussel species ( $R = 0.067$ ; 64<sup>th</sup> percentile). The intensity of these applications was moderate relative to other mussels in this study ( $I = 0.150$ ; 43<sup>rd</sup> percentile).

An analysis of substrate records found that 100% of all Lilliput occurrences were found within Types I and II habitat which resulted in the highest habitat preference value for all mussel species ( $H = 1$ ; tied with Eastern Pondmussel). The toxicity component of the relative risk equation was scored using Kidneyshell as a surrogate species, which resulted in a moderate toxicity value for Lilliput in relation to other mussels ( $T = 0.543$ ; 50<sup>th</sup> percentile).

The risk assessment found the overall relative risk value for Lilliput to be high in comparison to all other mussel species ( $RR_M = 0.005$ ; Figure 9). The overall risk value placed Lilliput in the 71<sup>st</sup> percentile for mussels, which was due to moderately high exposure to gB applications as well as its high toxicity value.

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## FAWNSFOOT

**Scientific Name:** *Truncilla donaciformis*

**Current COSEWIC Status and Year of Designation:** Endangered, April 2008

**SARA Schedule, Status and Year of Designation:** Schedule 1, Endangered, August 2019

**Current COSSARO Status and Year of Assessment:** Endangered, June 2009

### Distribution

Historically, Fawnsfoot was known from Lake Huron, Lake St. Clair, Lake Erie and some of their tributaries, as well as the Detroit and Niagara rivers (COSEWIC 2008a). Currently, its distribution is restricted to the Grand, Thames, and Sydenham rivers. A single individual has also been found in both the Saugeen River drainage and the St. Clair River delta (DFO 2011). Distribution in the Great Lakes for records since 1998 is provided in Figure A5.75.

### Allowable Harm

Allowable harm for Fawnsfoot has not been estimated because of a lack of data on population growth rates and projections. However, Young and Koops (2011a) predicted which vital rates were likely to be most sensitive to harm. They found that Fawnsfoot population growth is most sensitive to age at maturity, fecundity, and glochidial survival. Therefore, harm to these life-history stages should be minimized to avoid jeopardizing the survival and future recovery of Canadian populations. For more info information on Fawnsfoot allowable harm, see Young and Koops (2011a).

### Risk Associated with Granular Bayluscide

#### Background

No studies have examined the tolerance of the Fawnsfoot to Bayluscide exposure, so mortality information was based on its surrogate, the Kidneyshell. Further research is required to investigate if applying Bayluscide in areas where Fawnsfoot is known will have adverse effects on the species or its host fishes.

#### Relative Risk

This study found that gB applications in the Great Lakes have overlapped with Fawnsfoot locations in the past (Table 2, Figure A5.75). For example, approximately 13% of all ALIS segments within the range of Fawnsfoot have overlapped with a gB application location from 2011 to 2017 (Table 2), which represents less than 1% of all applications. This was a high spatial value in comparison to all other mussel species ( $R = 0.133$ ; 86<sup>th</sup> percentile). The intensity of applications was low in relation to other mussels in this study ( $I = 0.113$ ; 14<sup>th</sup> percentile; Figure 8).

Through the analysis of substrate records, approximately 67% of all Fawnsfoot occurrences were found within Types I and II habitat, which resulted in a low habitat preference value in comparison to all mussel species ( $H = 0.669$ ; 14<sup>th</sup> percentile). The toxicity component of the relative risk equation was scored using Kidneyshell as a surrogate species, which resulted in a high toxicity value for Fawnsfoot ( $T = 0.543$ ; 50<sup>th</sup> percentile).

The risk assessment found the overall relative risk value for Fawnsfoot to be high in comparison to other mussel species ( $RR_M = 0.005$ ; Figure 9). The overall risk value placed Fawnsfoot in the 79<sup>th</sup> percentile for mussels, which was largely due to high exposure to past gB applications.

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## RAYED BEAN

**Scientific Name:** *Villosa fabalis*

**Current COSEWIC Status and Year of Designation:** Endangered, April 2010

**SARA Schedule, Status and Year of Designation:** Schedule 1, Endangered, June 2003

**Current COSSARO Status and Year of Assessment:** Endangered, June 2010

### Distribution

In Canada, Rayed Bean was previously known from Lake Erie, the Detroit River, and the Sydenham and Thames rivers in the Lake St. Clair drainage. However, it is now restricted to the middle reach of the Sydenham River and a small section of the Thames River (COSEWIC 2010b). Distribution in the Great Lakes for records since 1998 is provided in Figure A5.76.

### Risk Associated with Granular Bayluscide

#### Background

No studies have examined the tolerance of the Rayed Bean to Bayluscide exposure, so mortality information for the risk assessment was based on its surrogate, the Kidneyshell (mortality of 54%, see Newton et al. 2017). Although the direct effects of Bayluscide exposure on mortality have not been tested, toxicity tests have been conducted on some of the host fish species for this mussel. For instance, toxicity to Bayluscide has been reported in the Largemouth Bass, a known host for Rayed Bean. Marking and Hogan (1967) reported an  $LC_{50}$  value of 0.062 ppm after 96 hours of exposure to Bayer 73 for this host species. A total of 18 Largemouth Bass were also found dead in three river deltas of Lake Champlain (Boquet, Ausable, and Salmon) after the application of Bayer 73 (5% granular) in 1991 and 1995 (Fisheries Technical Committee 1999). A reduction in available host fishes may result in less glochidia surviving to metamorphose into juveniles and, therefore, less recruitment into the population.

#### Relative Risk

This study found that gB applications in the Great Lakes have overlapped with Rayed Bean locations in the past (Figure A5.76). For example, approximately 4% of all ALIS segments within the range of Rayed Bean have overlapped with a gB application location from 2011 to 2017 (Table 2), which represents less than 1% of all applications. This was a moderate level of spatial overlap in comparison to other mussels in this study ( $R = 0.043$ ; 50<sup>th</sup> percentile). Measuring the intensity of these applications where they have occurred, revealed that the species scored the median value in this risk assessment category in relation to other mussels in this study ( $I = 0.200$ ; 50<sup>th</sup> percentile; Figure 8).

Through the analysis of substrate records, approximately 88% of all Rayed Bean occurrences were found within Types I and II habitat, which resulted in a high habitat preference value for the species in comparison to other mussels ( $H = 0.884$ ; 86<sup>th</sup> percentile). Furthermore, one gB application occurred within what is presently Rayed Bean critical habitat in 2012 (Table 3; Figure A5.77). The toxicity component of the relative risk equation was evaluated using Kidneyshell as a surrogate species, which resulted in a high toxicity value ( $T = 0.543$ ; 50<sup>th</sup> percentile).

The risk assessment found the overall relative risk value for Rayed Bean to be moderate in comparison to other mussel species ( $RR_M = 0.004$ ; Figure 9). The overall risk value placed Rayed Bean in the 57<sup>th</sup> percentile for mussels, which was largely due to median values in the spatial and intensity components of the risk assessment.



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## RAINBOW

**Scientific Name:** *Villosa iris*

**Current COSEWIC Status and Year of Designation:** Special Concern, November 2015

**SARA Schedule, Status and Year of Designation:** Schedule 1, Special Concern, August 2019

**Current COSSARO Status and Year of Assessment:** Special Concern, June 2016

### Distribution

In Canada, Rainbow occurs only in Ontario where it is found in the St. Clair River delta and the Saugeen, Maitland, Bayfield, Ausable, Sydenham, Thames, Grand, Trent, Salmon, and Moira rivers and various tributaries of each (DFO 2011, DFO unpublished data). It is presumed extirpated from Lake Erie (Long Point Bay, Rondeau Bay), and the Niagara, Detroit, and St. Clair rivers (DFO 2011). Distribution in the Great Lakes for records since 1998 is provided in Figure A5.78.

### Allowable Harm

Allowable harm for Rainbow has not been estimated because of a lack of data on population growth rates and projections. However, Young and Koops (2011a) predicted which vital rates were likely to be most sensitive to harm. In that study, Rainbow population growth was most sensitive to adult survival and somewhat sensitive to juvenile survival. Therefore, harm to these life-history stages should be minimized to avoid jeopardizing the survival and future recovery of Canadian populations. For more information on Rainbow allowable harm, see Young and Koops (2011a).

### Risk Associated with Granular Bayluscide

#### Background

A study by Boogaard et al. (2015) reported a mortality rate of approximately 14% for adult and 38% for sub-adult Rainbow after eight hours of exposure to gB at concentrations applied in the field (9.3 mg/L). Sub-lethal responses (defined as gaped valves, production of mucus, and/or foot extension outside the shell) were also much more delayed in adults, when compared to sub-adults (ET<sub>50</sub> of 271 minutes vs. 132 minutes) and adults were more likely to recover after displaying these responses (78% vs 41%). These data suggest that sub-adults are more sensitive to the effects of Bayluscide than adults. Indirect impacts as a result of exposure to Bayluscide include potential impacts to mussel host fishes. Of the known host fish species for Rainbow, toxicity to Bayluscide has been reported in Largemouth Bass and Yellow Perch. Marking and Hogan (1967) reported an LC<sub>50</sub> value of 0.062 ppm in Largemouth Bass and an LC<sub>50</sub> of 0.081 ppm in Yellow Perch after 96 hour of exposure to Bayer 73 (pH of 7.5). Mortality in the field has also been observed in both of these species after the application of Bayluscide (Fisheries Technical Committee 1999, M. Steeves, SLCC, unpublished data). A reduction in available host fishes may limit recruitment in the mussel population.

#### Relative Risk

This study found that gB applications in the Great Lakes have had little overlap with Rainbow locations in the past (Table 2, A5.79). For example, approximately 1% of all ALIS segments within the range of Rainbow have overlapped with a gB application location from 2011 to 2017 (Table 2), which represents 2% of all applications. This was the lowest level of spatial overlap in comparison to all other mussel species ( $R = 0.012$ ). The intensity of applications was among the highest for mussels ( $I = 0.733$ ; Figure 8).

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An analysis of substrate records indicated that approximately 82% of all Rainbow occurrences were found within Types I and II habitat. This resulted in a high habitat preference value for the species in comparison to other mussels ( $H = 0.818$ ; 71<sup>st</sup> percentile). Furthermore, it was found that 13 gB applications occurred within what was previously identified as Rainbow critical habitat (Proposed) from 2012-2017 (Table 3, Figure A5.80). The highest published mortality value for Rainbow (0.143) was used to evaluate toxicity, which resulted in a low value for Rainbow relative to all other mussel species (7<sup>th</sup> percentile).

The risk assessment found the overall relative risk value for Rainbow to be low in comparison to other mussel species ( $RR_M = 0.001$ ; Figure 9). The overall risk value placed Rainbow in the 29<sup>th</sup> percentile for mussels, which was largely due to its low exposure history to gB application as well as lower reported mortality compared to other mussels in the risk assessment.

## MITIGATION AND ALTERNATIVES

Estimates of Bayluscide-induced mortality (Smyth and Drake 2021) indicate that although the most likely outcome of an individual gB application cycle is zero or relatively low mortality of fishes and mussels of conservation concern, in some cases (i.e., 5% of the time), much higher mortality can occur on the order of ones to tens of at-risk fishes killed and potentially hundreds of Silver Lamprey, Northern Brook Lamprey, and freshwater mussels. Based on a worst-case scenario in which gB is applied annually and recovery from its effects does not occur, the 50–100 year effect of repeated Bayluscide application could reduce abundance by as much as 100% from baseline for some species (Northern Madtom, *Ichthyomyzon* spp.) or up to 90% for others (Eastern Sand Darter) if populations are small, but would be less severe if populations are large. Smyth and Drake (2021) also reported that modifying the size and number of application sites can influence the likelihood of extreme mortality events, but will have little effect on the average outcome of an application cycle. Although Smyth and Drake (2021) evaluated mortality for only a subset of tributaries (Detroit, St. Clair, Thames, Sydenham rivers), results of the relative risk assessment in this document indicate that the conditions for exposure and mortality exist throughout the Great Lakes basin and are not limited to the four focal rivers. Therefore, mitigation measures and alternatives may be warranted to reduce the likelihood of direct Bayluscide-induced mortality on species of conservation concern. Moreover, because very little information is available about other pathways of effects (i.e., indirect pathways involving food-web effects; non-physiological mechanisms such as avoidance), mitigation of those pathways may also be warranted.

The level of risk mitigation is a management decision that involves evaluating the benefits and consequences associated with mitigations and alternatives. Benefits include the potential to reduce mortality and other Bayluscide-induced changes to species of conservation concern, whereas consequences include the potential for reduced effectiveness of Sea Lamprey assessment and control, which in some cases, may result in increased Sea Lamprey predation on species such as Lake Sturgeon.

Mitigation typically involves defining a desired level of protection, evaluating the likelihood of success of a given mitigation measure, and monitoring its effectiveness if implemented. Mitigation hierarchies are commonly applied to reduce the effects of human activities on biodiversity and species of conservation concern (DFO 2015, Tallis et al. 2015, Squires and Garcia 2018). Mitigation hierarchies typically recommend avoiding the ecosystem in question as the initial conservation action. Avoidance is recommended because it is perfectly protective of the ecosystem component(s) in question, and therefore, eliminates uncertainty about how the ecosystem will respond. When avoidance is not possible, mitigations and alternatives may be pursued, followed by offsetting measures if mitigations cannot be applied.

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Below, a qualitative review of potential mitigation and alternative measures (Table 4) is provided focusing on potential benefits and key uncertainties and considerations for species of conservation concern. In some cases, mitigation measures have been evaluated in Smyth and Drake (2021). In other cases, Table 4 presents measures that have not been quantitatively evaluated and require further research. If mitigation measures are pursued in the field, rigorous testing and evaluation is recommended to ensure that desired benefits are realized following implementation.

Table 4. Mitigations and alternatives to granular Bayluscide (gB) application in the Great Lakes basin, focusing on benefits and considerations for species of conservation concern.

Action	Benefit to Species of Conservation Concern	Considerations
Avoidance of watersheds containing species of conservation concern	Avoidance removes all negative direct and indirect, physiological and non-physiological mechanisms that lead to reduced viability of fishes and mussels of conservation concern.	<ul style="list-style-type: none"> <li>• Avoidance may lead to reduced effectiveness of Sea Lamprey assessment and control, which may result in negative effects for species that experience predation or wounding from Sea Lamprey (e.g., Lake Sturgeon).</li> <li>• The trade-off between avoidance and positive and negative effects of gB application among fishes and mussels of conservation concern is poorly understood (e.g., avoidance may benefit most species of conservation concern while negatively affecting those vulnerable to Sea Lamprey predation like Lake Sturgeon). These issues may be of lesser importance in watersheds that lack species susceptible to Sea Lamprey predation.</li> </ul>
Reducing realized concentrations of gB in the aquatic environment	Potential to reduce mortality and other direct and indirect pathways (including sub-lethal effects) to fishes and mussels of conservation concern.	<ul style="list-style-type: none"> <li>• The maximum concentration at which toxicity is negligible for non-target species is unknown given multiple plausible direct and indirect pathways.</li> <li>• Requires large investment and research effort to evaluate effectiveness on non-target species.</li> <li>• Uncertainty exists about the fate of gB in the aquatic environment at current application rates, which would need to be resolved to demonstrate a meaningful reduction in realized concentration.</li> <li>• The trade-off between reducing realized concentrations and positive and negative effects among fishes and mussels is poorly understood, particularly for species experiencing Sea Lamprey predation like Lake Sturgeon.</li> </ul>
Reducing the frequency with which gB is applied in a particular area	Potential to reduce mortality and other direct and indirect pathways (including sub-lethal effects) to fishes and mussels of conservation concern.	<ul style="list-style-type: none"> <li>• Relationship between application frequency and population effects is non-linear and highly dependent on assumed population abundances (see Smyth and Drake [2021]), which are poorly understood for most species of conservation concern.</li> <li>• The trade-off between reducing the frequency of application and positive and negative effects among fishes and mussels is poorly understood, particularly for species experiencing Sea Lamprey predation like Lake Sturgeon.</li> </ul>
Reducing size or number of gB application sites	Potential to reduce mortality, especially rare, high-abundance mortality events (Smyth and Drake 2021). Potential to reduce other direct and indirect pathways (including sub-lethal effects) to fishes and mussels of conservation concern.	<ul style="list-style-type: none"> <li>• Does not eliminate the risk of mortality to fishes and mussels of conservation concern.</li> <li>• Relationship between application site size/number and mortality is non-linear (Smyth and Drake 2021).</li> <li>• The trade-off between reducing size/number of gB application sites and positive and negative effects among fishes and mussels is poorly understood, particularly for species experiencing Sea Lamprey predation like Lake Sturgeon.</li> </ul>

Action	Benefit to Species of Conservation Concern	Considerations
Move location of application sites to areas outside or downstream of critical habitat	Potential to decrease direct and indirect pathways to fishes and mussels of conservation concern, particularly when applications are located downstream of occupied habitat.	<ul style="list-style-type: none"> <li>• The distribution of fishes and mussels of conservation concern is poorly known; assumes range boundaries known with precision.</li> <li>• May not reduce indirect effects (requires better understanding of food web linkages).</li> </ul>
Salvage/exclusion of mussels or fishes of conservation concern prior to gB application.	Decreases the number of fishes and mussels of conservation concern within application area. Potential to reduce direct and indirect pathways.	<ul style="list-style-type: none"> <li>• Removal sampling and salvage is often incomplete due to gear selectivity; fishes and mussels of conservation concern are likely to remain in application site and experience gB exposure.</li> <li>• Deepwater mussels are extremely challenging to sample and relocate</li> <li>• Potential mortality or harm to fishes and mussels can occur during capture and relocation (e.g., consequences for growth or survival).</li> <li>• Mobile species can return to application area prior to gB treatment.</li> </ul>
Offset impacts to non-target species through habitat restoration or other feasible means	An offset such as habitat restoration may increase the availability or quality of habitat, thereby increasing the viability of non-target fishes or mussels	<ul style="list-style-type: none"> <li>• Effectiveness of offsetting for fishes and mussels of conservation concern is highly uncertain. Certainty can be increased by implementing offset in advance and validating effectiveness.</li> <li>• Species in question may not be habitat limited, so habitat-related offsets may not provide benefit to species. Feasible offsets may not exist for the species in question.</li> <li>• Physical habitat manipulations may be insufficient to produce net benefit for species if the application of gB has the potential to extirpate fishes or mussels from the system.</li> </ul>
Application of gB after Aug 1 <sup>st</sup> or seasonally outside of reproductive periods for a given species	Avoids harm to sensitive life stages (e.g., spawning, YOY) for many fish and mussel species	<ul style="list-style-type: none"> <li>• Does not eliminate the risk of mortality to fishes and mussels of conservation concern.</li> <li>• Currently a lack of knowledge about how the timing of application leads to mortality or other effects on fishes and mussels.</li> <li>• Unknown if seasonal adjustment of application imposes other trade-offs or unexpected consequences.</li> </ul>

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## CONCLUSIONS AND SOURCES OF UNCERTAINTY

This research document and accompanying modelling document (Smyth and Drake 2021) have evaluated the ecological risk of gB applications for fishes and mussels of conservation concern in the Canadian waters of the Great Lakes basin. Smyth and Drake (2021) identified the absolute risk of gB application in four river systems in southern Ontario, whereas this document identified relative risk across Canadian waters of the Great Lakes basin.

Both research documents have led to significant insights about the effect of gB applications on fishes and mussels of conservation concern in the Great Lakes basin. Primary findings indicate that: 1) a gradient of relative risk exists among species based on gB application locations, habitat features, and toxicity; 2) although gB applications occurred within the range of 36 fish and mussel species of conservation concern (including within areas currently or previously identified as critical habitat for 6 fishes and 10 mussels) in the Great Lakes region from 2011 to 2017, gB exposure is relevant for less than 30% of a species' range, and for less than 30% of gB application sites; 3) in most cases, gB applications are not expected to pose direct mortality, but high mortality events are possible under certain conditions, especially for native lampreys and freshwater mussels; and, 4) mitigation measures such as changes to application site size and number have model-based support to reduce the likelihood of extreme mortality events.

In most cases, the results from Smyth and Drake (2021) align with results from this study. For example, the relative risk assessment demonstrated that native lampreys (*Ichthyomyzon* spp.) exhibited the greatest relative risk among fishes. This finding was consistent with Smyth and Drake (2021) who indicated that native lampreys can experience very high mortality events (among the highest of all species considered) as a result of gB applications under certain conditions. Excluding native lampreys, relative risk for SARA-listed fishes was highest for Northern Madtom. This was consistent with findings from Smyth and Drake (2021), where analyses demonstrated that gB applications can significantly affect Northern Madtom populations depending on the application frequency and the area of occupied critical habitat. Similarly for freshwater mussel species, consistent results between this study and Smyth and Drake (2021) were found in terms of the overall risk rankings for Fawnsfoot, Mapleleaf, and Rainbow, and the estimated absolute mortalities for these species in focal rivers in southern Ontario. Low absolute mortality estimates for Rainbow and Mapleleaf in the Sydenham River (Smyth and Drake 2021) were consistent with their lower risk rankings compared to other mussel species here. Likewise, absolute mortality (95<sup>th</sup> percentile) for Fawnsfoot in the Thames River ranked 3<sup>rd</sup> highest among mussels, which again was consistent with Fawnsfoot ranking 4<sup>th</sup> highest among mussels in this study.

Some inconsistencies occurred between risk rankings from this paper and mortality estimates from Smyth and Drake (2021) that could be related to methodology. In Smyth and Drake (2021), mortality was estimated in four focal systems where likelihood of occurrence was based on the probability that a species would be found in Type I or Type II habitats. Whereas, habitat use in this risk assessment was identified as the percentage of occurrences in Types I and II habitats across the Great Lakes region. Furthermore, absolute mortality estimates considered species densities, whereas this study did not factor abundance into the overall risk calculation.

Ultimately, the overall relative risk value does not equate to an absolute mortality event resulting from a gB application (as estimated in Smyth and Drake 2021) and does not consider a species' patchiness nor density. Inconsistencies include Lake Sturgeon's high overall risk here, yet Smyth and Drake (2021) estimated zero mortality under all scenarios modeled for the species in the Detroit and St. Clair rivers. Multiple factors likely played a role in this discrepancy, including its low population density and removal of offshore records from this risk assessment.

Inconsistencies were not limited to fishes. In some cases, as absolute mortality estimates for

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mussels in Smyth and Drake (2021) contradicted relative risk rankings in this paper. For example, Smyth and Drake (2021) estimated zero mortality (95<sup>th</sup> percentile value) for Salamander Mussel and Threehorn Wartyback, yet these two species ranked highest in the relative risk assessment. The discrepancy can be explained by the way in which risk was evaluated in both documents. Relative risk was a function of the interaction between the location and frequency of gB application, species' distributions, habitat associations that predispose species to exposure and toxicity to the compound, but did not include species density as an assessment variable. Species density was incorporated within Smyth and Drake (2021) and the zero mortality estimated in that document was driven by extremely low species densities (i.e., very few individuals found during surveys where density could be calculated) as well as other system-specific factors. Overall, these results suggest that encountering the species during gB application is very unlikely, but mortality may be substantial if this occurs within an occupied habitat patch.

Although this research document and Smyth and Drake (2021) indicate non-zero ecological risk for many species of conservation concern, untransformed relative risk values did not approach 1.0. This suggests that even for species with highest relative risk, species have refuge capacity via ranges that are not completely overlapped by gB applications, occupy habitats that are not solely the focus of gB applications, and/or have at least some buffering capacity to the toxicity of the compound. Further work is required to relate relative risk to absolute mortality for each species.

Despite increased knowledge about the potential effects of gB, several uncertainties exist that have influenced the scope for relative risk (this document) and the direct mortality of fishes and mussels (Smyth and Drake 2021). These are related to data limitations for fishes and mussels of conservation concern, uncertainties about gB toxicity and the appropriateness of surrogate species, uncertainties about how non-target species interact with gB applications (e.g., avoidance and its consequences), and the effectiveness of potential mitigation measures if pursued. These are elaborated on below.

First, incomplete distribution data presented challenges for assessing the relative risk of some species of conservation concern. Data limitations were particularly relevant for mussels in the St. Clair and Detroit rivers where lack of sampling in recent decades led to uncertainty about whether extant populations exist in those systems. Field data from 2019 revealed that mussel species at risk do inhabit the Detroit River but these data were not available for the analysis (Allred et al. 2020). Furthermore, gaps in the distribution of Lake Sturgeon for parts of the St. Clair and Detroit rivers in areas where they are known to occur presented challenges when evaluating relative risk.

Second, models from Smyth and Drake (2021) indicated that the long-term population consequences of Bayluscide-induced mortality depend heavily on assumed population sizes of non-target fishes and mussels. However, for most species of conservation concern, population abundance is unknown. To illustrate the consequences of Bayluscide-induced mortality across 50 or 100 years under a range of assumed population sizes, Smyth and Drake (2021) used an extrapolation approach based on patch-specific species densities and assumptions about the proportion of the bounded range that was occupied. The challenge of estimating patch density is described in Smyth and Drake (2021). Gaining knowledge of true population abundance would significantly refine 50- and 100-year population consequences and would allow the relative risk formulation in this document to be revised.

Third, although gB is applied at a constant rate to achieve a peak concentration of 11 mg/L (9.3 mg/L active ingredient niclosamide) in the bottom 5 cm of the water column (Adair and Sullivan 2004), it is likely that variability in environmental conditions (e.g., river discharge,

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habitat complexity) lead to variability in the environmental concentration of the compound. Refined in-water estimates of gB concentrations, including the effect of flow, depth, distance from application site and other water quality variables (e.g., temperature, pH, conductivity), is needed to refine the likelihood of mortality. Also, as associations with Type I and II habitat predispose fish and mussel species to direct exposure to gB, any disconnect between how field programs identify substrate (or whether substrate is homogenous throughout an application) will have large bearing on risk assessment scores. Also, almost all toxicity information in this document is based on surrogate values through taxonomic matching. For many species, the closest surrogate species used to determine toxicity to gB does not belong to the same genus (see Table 1) and species-specific differences in tolerance are likely to occur. This issue becomes of even greater importance for species such as Lake Sturgeon and Spotted Gar where toxicity information with surrogates was obtained from different subclasses, and infraclasses, respectively. Finally, relative risk was based on spatial and temporal patterns of past gB applications. New application patterns into the future would change relative risk and may require a revision of the spatial and intensity values used in this risk assessment (habitat and toxicity values would remain unchanged). As the future dynamics of Sea Lamprey in the Great Lakes basin are unknown, changes in the ecosystems requiring Sea Lamprey assessment is not known with certainty.

In general, non-physiological mechanisms (e.g., gB avoidance and its consequences) are poorly known and were not incorporated within the relative risk assessment. Evidence exists that some surrogate species may detect and avoid gB by elevating their position in the water column during laboratory trials (Boogaard et al. 2016b), but it is unclear whether at-risk species would display similar responses. It is also unclear how these responses would differ (if at all) in a field setting or what the consequences are of avoidance across the duration and spatial extent of an application cycle. Although avoidance may reduce the direct mortality pathway, displacement of species to suboptimal habitat may impair growth or survival. In the case of small bodied fishes, the ability to avoid large application areas may simply not be feasible due to poor swimming ability. Mussels do not have the ability to avoid gB through movement, but may use valve-closure as an avoidance mechanism, thereby reducing filtering and resulting processes (e.g., feeding, excretion). The consequences of avoidance behaviours are unknown as they relate to potential effects on growth and survival for fishes and mussels.

Incomplete knowledge of food-web connections also exists for most species of conservation concern making it extremely difficult to gauge the importance of indirect pathways relative to direct mortality. For some species, indirect pathways may be highly relevant for eliciting population responses such as for species with obligate species dependencies (e.g., small-bodied host fishes for freshwater mussels). Importantly, food web effects can promote beneficial outcomes for species at risk such as relaxing predation pressure on small fishes by reducing the abundance of non-lamprey predators or via direct rescue from lamprey-induced predation or wounding (e.g., Lake Sturgeon). All factors identified above (increased knowledge of population abundance, environmentally relevant concentrations of gB, species-specific toxicity, likelihood and consequences of avoidance across sessile and non-sessile organisms, indirect physiological and non-physiological food web effects) are important avenues for future research.

Lastly, this document presents several mitigation measures, ranging from avoidance to offsetting, which may reduce the potential for Bayluscide-induced mortality on fishes and mussels of conservation concern. Should these mitigation measures be pursued, it is recommended that they be accompanied by rigorous field testing to ensure intended benefits are realized and unintended outcomes minimized. More broadly, additional analytical research may be needed to understand how proposed mitigation measures may reduce the efficacy of



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Sea Lamprey assessment, including how to maximize benefits to species of conservation concern while minimizing unintended consequences for Sea Lamprey assessment and control.

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**APPENDIX 1. NUMBER OF GRANULAR BAYLUSCIDE (GB) APPLICATIONS THAT HAVE OCCURRED SINCE 2011 NEAR RECORDS OF SPECIES OF CONSERVATION CONCERN IN ONTARIO. FISH AND MUSSEL RECORDS INCLUDE THOSE FROM 1998 ONWARDS.**

Species	Number of gB applications within:				Number of SAR sites within:		
	250 m of a SAR record	1000 m of a SAR record	2500 m of a SAR record	SAR Distribution (250 m buffered ALIS segments)	250 m of gB	1000 m of gB	2500 m gB
American Eel	17	95	128	120	16	58	100
Black Redhorse	13	26	35	31	6	6	9
Blackstripe Topminnow	2	6	6	15	6	17	27
Bridle Shiner	1	2	4	4	1	5	7
Channel Darter	29	100	196	193	12	35	48
Cutlip Minnow	0	0	0	0	0	0	0
Eastern Sand Darter	5	5	6	10	235	248	399
Grass Pickerel	6	20	29	39	5	17	180
Lake Chubsucker	1	6	9	10	1	33	157
Lake Sturgeon	4	29	65	81	5	19	47
Northern Brook Lamprey	14	52	99	118	4	5	8
Northern Brook Lamprey + Ichthyomyzon sp.	253	408	463	415	117	128	148

Species	Number of gB applications within:				Number of SAR sites within:		
	250 m of a SAR record	1000 m of a SAR record	2500 m of a SAR record	SAR Distribution (250 m buffered ALIS segments)	250 m of gB	1000 m of gB	2500 m gB
Northern Madtom	42	148	215	226	45	90	167
Northern Sunfish	11	57	97	86	23	50	72
Pugnose Minnow	1	1	3	12	1	1	3
Pugnose Shiner	5	21	61	56	6	34	199
Redside Dace	0	0	0	0	0	0	0
River Darter	0	1	1	6	0	6	6
River Redhorse	8	52	96	61	6	22	28
Silver Chub	0	0	0	0	0	0	0
Silver Lamprey	37	202	413	307	16	77	182
Silver Lamprey + Ichthyomyzon sp.	264	504	634	536	129	200	322
Silver Shiner	1	4	4	4	1	1	1
Spotted Gar	2	8	9	5	1	5	14
Spotted Sucker	43	116	181	193	55	102	161
Warmouth	5	9	9	5	1	20	78
Eastern Pondmussel	10	23	31	34	4	6	6

Species	Number of gB applications within:				Number of SAR sites within:		
	250 m of a SAR record	1000 m of a SAR record	2500 m of a SAR record	SAR Distribution (250 m buffered ALIS segments)	250 m of gB	1000 m of gB	2500 m gB
Fawnsfoot	1	4	4	9	4	4	6
Hickorynut	0	0	5	4	0	0	3
Kidneyshell	0	0	0	1	0	0	0
Lilliput	4	4	4	6	2	3	5
Mapleleaf	13	14	14	11	8	12	38
Northern Riffleshell	0	0	0	4	0	0	0
Rainbow	0	5	56	44	0	3	8
Rayed Bean	0	0	0	4	0	0	0
Round Hickorynut	0	0	0	4	0	0	0
Round Pigtoe	0	0	0	1	0	0	0
Salamander Mussel	0	0	1	4	0	0	1
Snuffbox	0	0	0	4	0	0	0
Threehorn Wartyback	1	4	4	8	4	4	6
Wavyrayed Lampmussel	1	4	4	8	1	1	1

**APPENDIX 2. THE NUMBER OF LARVAL SEA LAMPREY OCCURRENCES THAT HAVE OCCURRED NEAR RECORDS OF SPECIES OF CONSERVATION CONCERN IN ONTARIO. FISH AND MUSSEL RECORDS FROM 1998 ONWARDS.**

Species	Number of Sea Lamprey ammocoete records within:			
	250 m of a SAR record	1000 m of a SAR record	2500 m of a SAR record	SAR Distribution (250 m buffered ALIS segments)
American Eel	22	50	65	58
Black Redhorse	4	7	7	7
Blackstripe Topminnow	0	0	0	3
Bridle Shiner	0	0	6	0
Channel Darter	8	43	68	64
Eastern Sand Darter	7	7	8	8
Grass Pickerel	1	3	3	7
Lake Chubsucker	0	2	2	3
Lake Sturgeon	7	18	52	46
Northern Brook Lamprey	14	29	57	65
Northern Brook Lamprey + Ichthyomyzon sp.	178	226	286	252
Silver Lamprey	44	109	219	138
Silver Lamprey + Ichthyomyzon sp.	192	278	382	303
Northern Madtom	17	53	79	59
Northern Sunfish	0	20	35	31
Pugnose Minnow	0	0	0	3
Pugnose Shiner	1	7	28	25

Species	Number of Sea Lamprey ammocoete records within:			
	250 m of a SAR record	1000 m of a SAR record	2500 m of a SAR record	SAR Distribution (250 m buffered ALIS segments)
Redside Dace	4	20	55	30
River Darter	0	0	0	0
River Redhorse	2	16	37	25
Silver Shiner	3	4	16	15
Spotted Gar	0	0	2	0
Spotted Sucker	12	29	51	54
Warmouth	0	2	2	0
Eastern Pondmussel	7	12	20	16
Fawnsfoot	0	0	0	0
Hickorynut	0	0	1	1
Lilliput	0	0	1	0
Kidneyshell	0	0	0	0
Mapleleaf	0	0	0	0
Northern Riffleshell	0	0	0	0
Rainbow	0	17	24	20
Rayed Bean	0	0	0	0
Round Hickorynut	0	0	0	0
Round Pigtoe	0	0	0	0
Salamander Mussel	0	0	0	0
Snuffbox	0	0	0	0
Threehorn Wartyback	0	0	0	0
Wavyrayed Lampmussel	0	0	0	0

**APPENDIX 3. RESULTS OF THE RELATIVE RISK ASSESSMENT FOR FISH SPECIES OF CONSERVATION CONCERN TO BAYLUSCIDE APPLICATIONS**

Species		Spatial Score (R)	Intensity Score (I)	Habitat Preference Score (H)	Toxicology Score (T)	Overall Score (RRm)
Common Name	Scientific Name					
American Eel	<i>Anguilla rostrata</i>	0.088	0.353	0.771	0.632	0.0152
Black Redhorse	<i>Moxostoma duquesnei</i>	0.040	0.517	0.522	0.139	0.0015
Blackstripe Topminnow	<i>Fundulus notatus</i>	0.056	0.188	0.522	0.035	0.0002
Bridle Shiner	<i>Notropis bifrenatus</i>	0.014	0.100	0.970	0.035	0.0000
Channel Darter	<i>Percina copelandi</i>	0.148	1.000	0.667	0.046	0.0046
Cutlip Minnow	<i>Exoglossum maxillingua</i>	0.000	0.000	0.200	0.035	0.0000
Eastern Sand Darter	<i>Ammocrypta pellucida</i>	0.017	0.125	0.693	0.046	0.0001
Grass Pickerel	<i>Esox americanus vermiculatus</i>	0.031	0.163	0.742	0.046	0.0002
Lake Chubsucker	<i>Erimyzon sucetta</i>	0.020	0.100	0.944	0.139	0.0003
Lake Sturgeon	<i>Acipenser fulvescens</i>	0.261	0.338	0.730	0.532	0.0342
Northern Madtom	<i>Noturus stigmosus</i>	0.216	0.538	0.480	0.532	0.0298
Northern Sunfish	<i>Lepomis peltastes</i>	0.077	0.205	0.834	0.076	0.0010
Pugnose Minnow	<i>Opsopoeodus emiliae</i>	0.143	0.200	0.838	0.035	0.0008
Pugnose Shiner	<i>Notropis anogenus</i>	0.024	0.280	0.937	0.035	0.0002
Redside Dace	<i>Clinostomus elongatus</i>	0.000	0.000	0.833	0.035	0.0000
River Darter	<i>Percina shumardi</i>	0.222	0.150	0.545	0.046	0.0008
River Redhorse	<i>Moxostoma carinatum</i>	0.122	0.508	0.400	0.139	0.0035
Silver Chub	<i>Macrhybopsis storeriana</i>	0.000	0.000	1.000	0.035	0.0000
Silver Shiner	<i>Notropis photogenis</i>	0.018	0.100	0.215	0.035	0.0000
Spotted Gar	<i>Lepisosteus oculatus</i>	0.048	0.042	0.637	0.046	0.0001
Spotted Sucker	<i>Minytrema melanops</i>	0.174	0.386	0.717	0.139	0.0067
Warmouth	<i>Lepomis gulosus</i>	0.039	0.042	0.783	0.076	0.0001
Northern Brook Lamprey	<i>Ichthyomyzon fossor</i>	0.097	0.843	1.000	0.972	0.0797
Northern Brook Lamprey + Ichthyomyzon sp.	<i>Ichthyomyzon fossor</i> + <i>Ichthyomyzon</i> sp.	0.185	0.432	1.000	0.972	0.0776
Silver Lamprey	<i>Ichthyomyzon unicuspis</i>	0.246	0.439	1.000	0.972	0.1051
Silver Lamprey + Ichthyomyzon sp.	<i>Ichthyomyzon unicuspis</i> + <i>Ichthyomyzon</i> sp.	0.251	0.308	1.000	0.972	0.0751



**APPENDIX 4. RESULTS OF THE RISK ASSESSMENT FOR MUSSEL SPECIES OF CONSERVATION CONCERN TO BAYLUSCIDE APPLICATIONS**

Species		Spatial Score (R)	Intensity Score (I)	Habitat Preference Score (H)	Toxicology Score (T)	Overall Score (RRm)
Common Name	Scientific Name					
Eastern Pondmussel	<i>Ligumia nasuta</i>	0.045	0.425	1.000	0.155	0.0030
Fawnsfoot	<i>Truncilla donaciformis</i>	0.133	0.113	0.669	0.543	0.0055
Hickorynut	<i>Obovaria olivaria</i>	0.167	0.200	0.840	0.233	0.0065
Lilliput	<i>Toxolasma parvum</i>	0.067	0.150	1.000	0.543	0.0054
Kidneyshell	<i>Ptychobranhus fasciolaris</i>	0.023	0.050	0.776	0.543	0.0005
Mapleleaf	<i>Quadrula quadrula</i>	0.036	0.138	0.777	0.033	0.0001
Northern Riffleshell	<i>Epioblasma rangiana</i>	0.031	0.200	0.814	0.543	0.0028
Rainbow	<i>Villosa iris</i>	0.012	0.733	0.818	0.143	0.0011
Rayed Bean	<i>Villosa fabalis</i>	0.043	0.200	0.884	0.543	0.0042
Round Hickorynut	<i>Obovaria subrotunda</i>	0.077	0.200	0.667	0.444	0.0046
Round Pigtoe	<i>Pleurobema sintoxia</i>	0.016	0.050	0.748	0.224	0.0001
Salamander Mussel	<i>Simpsonaias ambigua</i>	0.167	0.200	0.706	0.543	0.0128
Snuffbox	<i>Epioblasma triquetra</i>	0.033	0.200	0.659	0.543	0.0024
Threehorn Wartyback	<i>Obliquaria reflexa</i>	0.130	0.133	0.763	0.543	0.0072
Wavyrayed Lampmussel	<i>Lampsilis fasciola</i>	0.016	0.133	0.768	0.508	0.0008

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## **APPENDIX 5. DISTRIBUTION OF SPECIES OF CONSERVATION CONCERN IN RELATION TO LARVAL SEA LAMPREY OCCURRENCES AND BAYLUSCIDE APPLICATION SITES**

Maps for fish and mussel Species at Risk (SAR) and larval Sea Lamprey distributions are shown in cases where both species co-occur, defined as a Sea Lamprey record found within 250 m of a SAR Aquatic Landscape Inventory System (ALIS) segment or within 2500 m of a SAR record; maps of granular Bayluscide (gB) within the distribution of fish or mussel species are shown where applications occur within 250 m of a SAR ALIS segment, or within 2500 m of a SAR record; and, maps of gB applications within the critical habitat of fish or mussel species are shown where a gB application occurred within areas delineated as critical habitat as published in final or proposed recovery documents on the SARA registry.

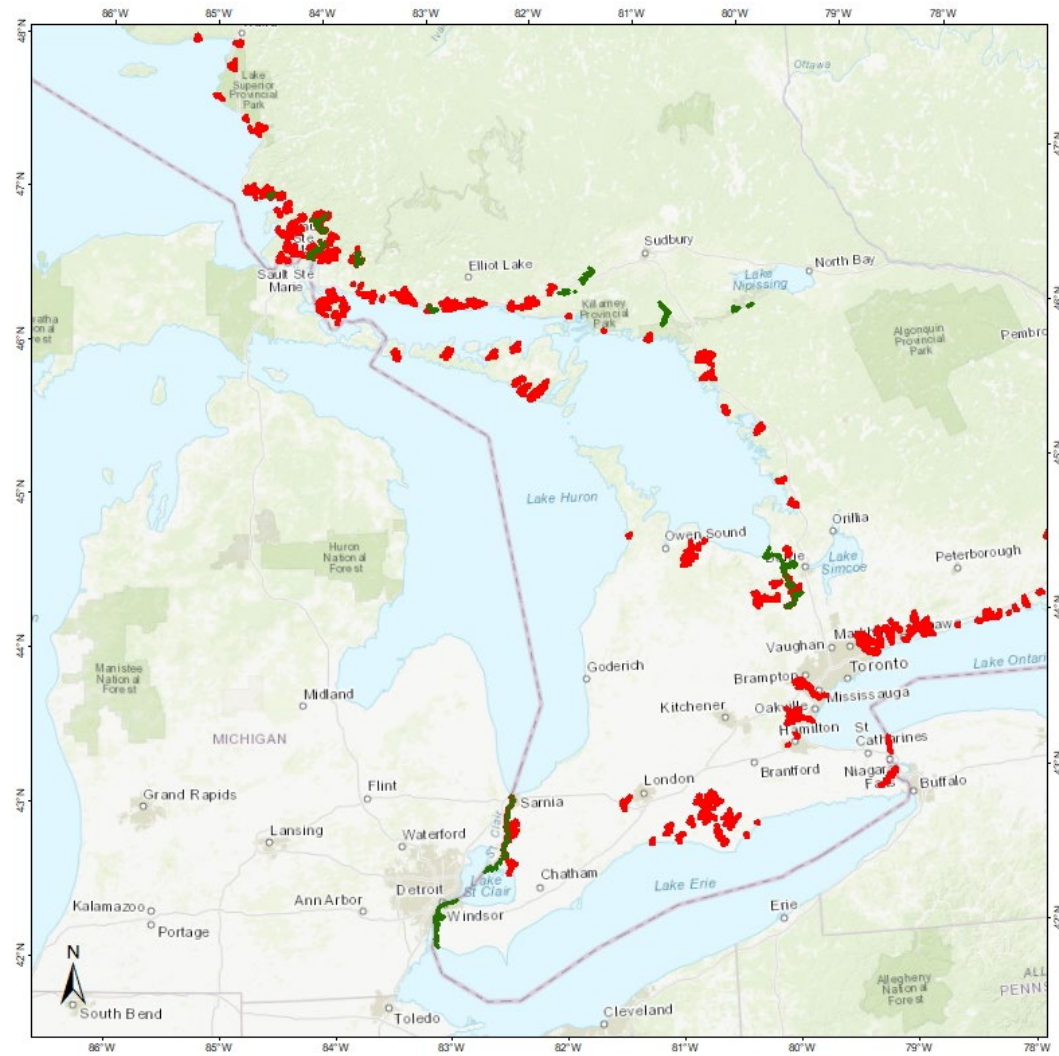


Figure A5.1 Spatial distribution of Lake Sturgeon (1998–2017; green shaded area) and larval Sea Lamprey (2011–2017; red shaded area) in Ontario’s Great Lakes region.

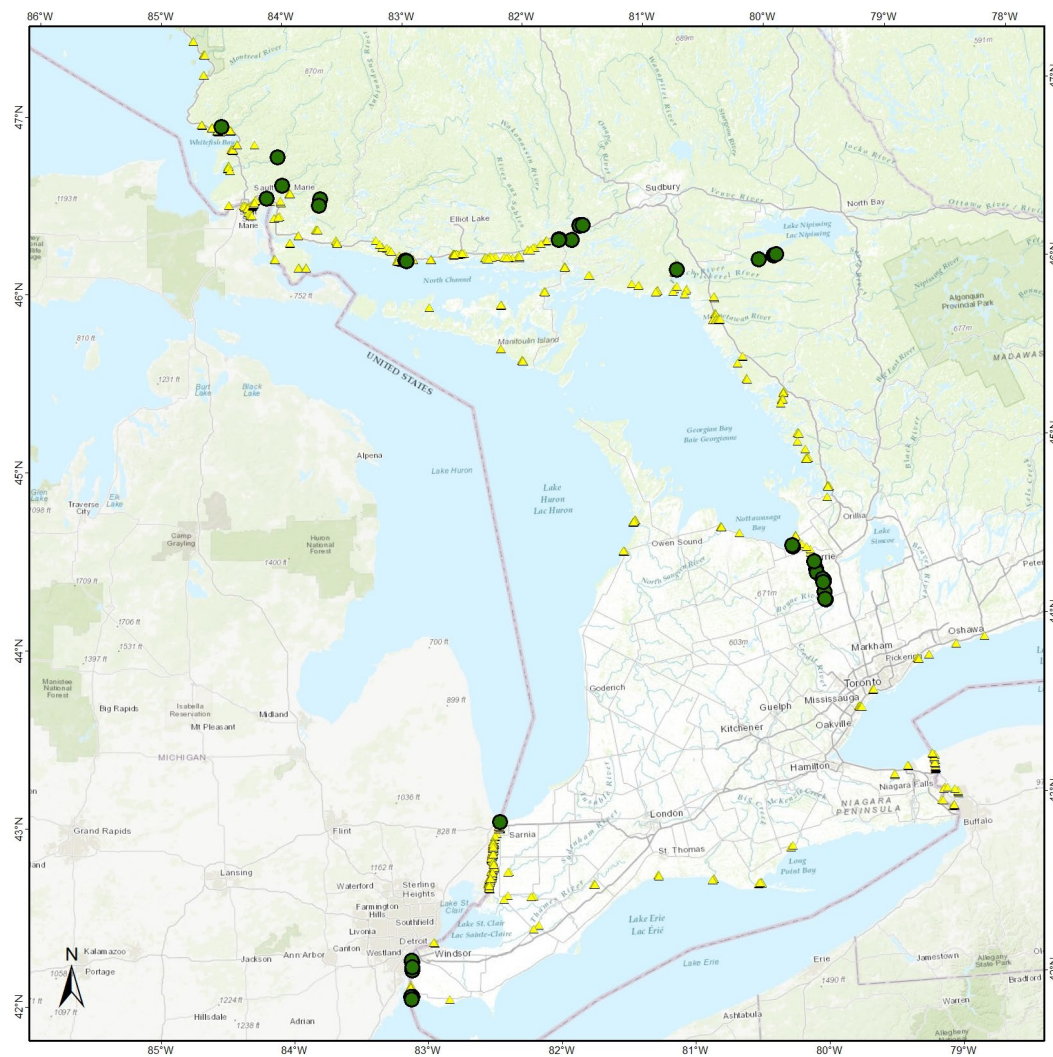


Figure A5.2. Spatial distribution of Lake Sturgeon records (1998–2017; green circles) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.

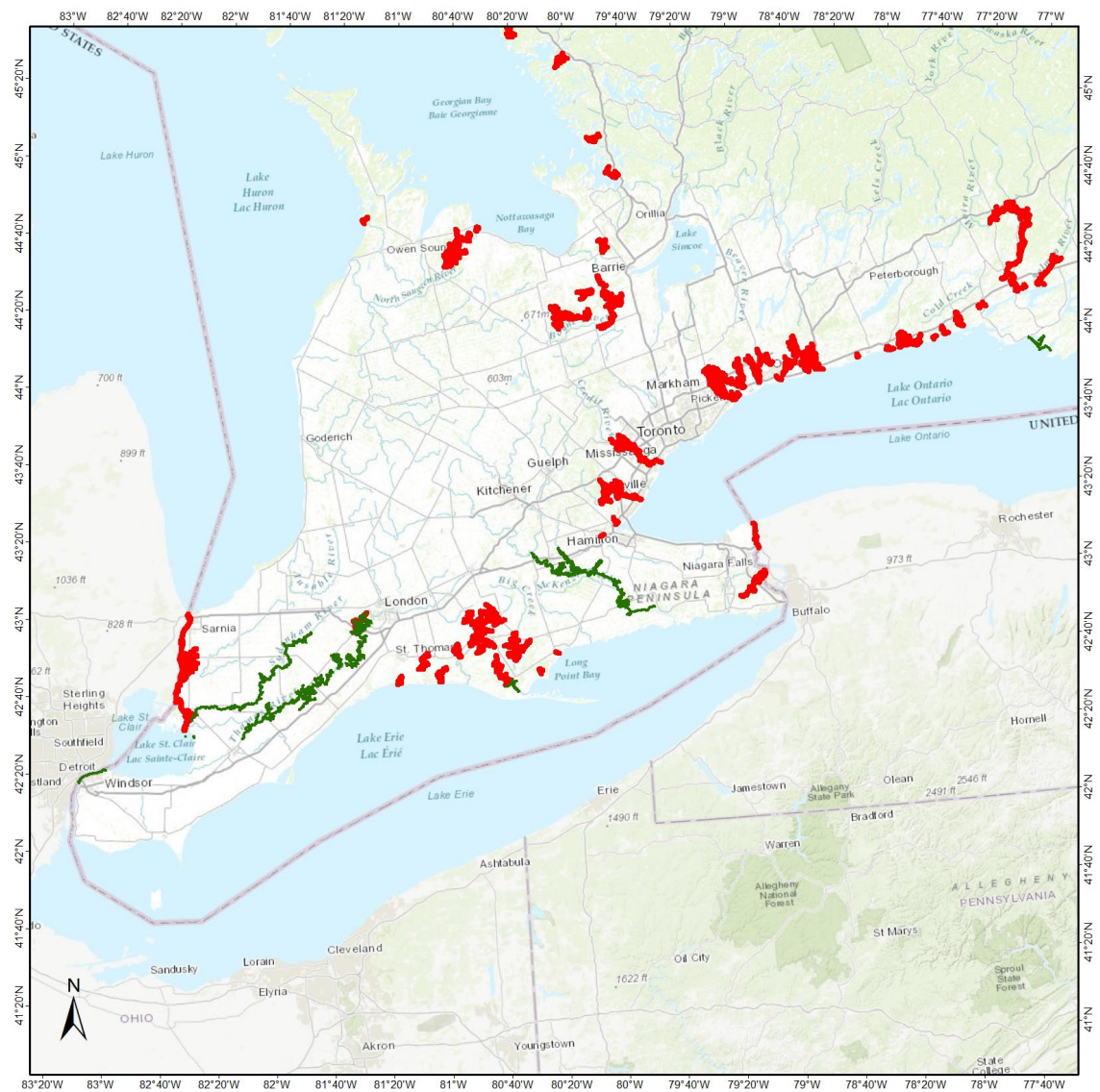


Figure A5.3. Spatial distribution of Eastern Sand Darter (1998–2017; green lines) and larval Sea Lamprey (2011–2017; red lines) in Ontario's Great Lakes region.



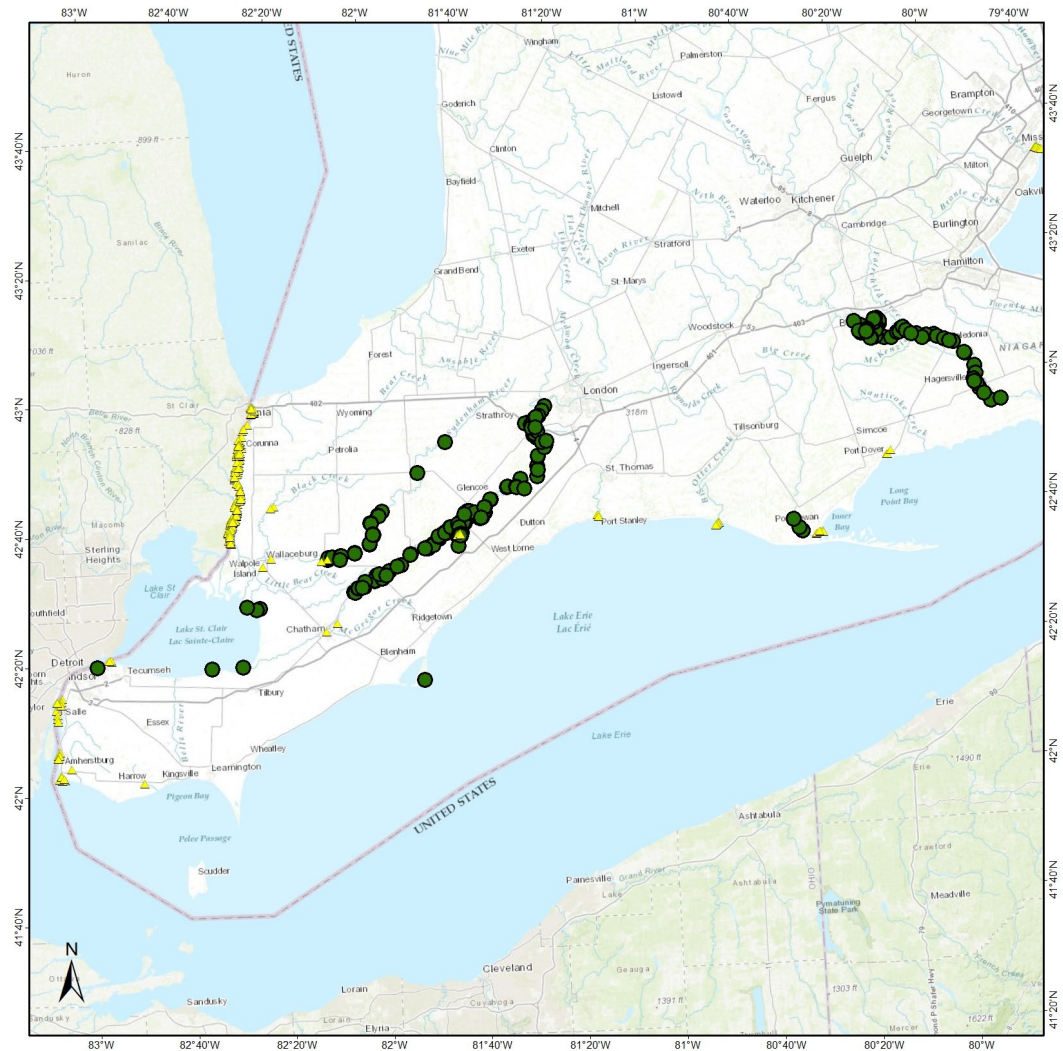


Figure A5.4. Spatial distribution of Eastern Sand Darter (1998–2017; green circles) records and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.

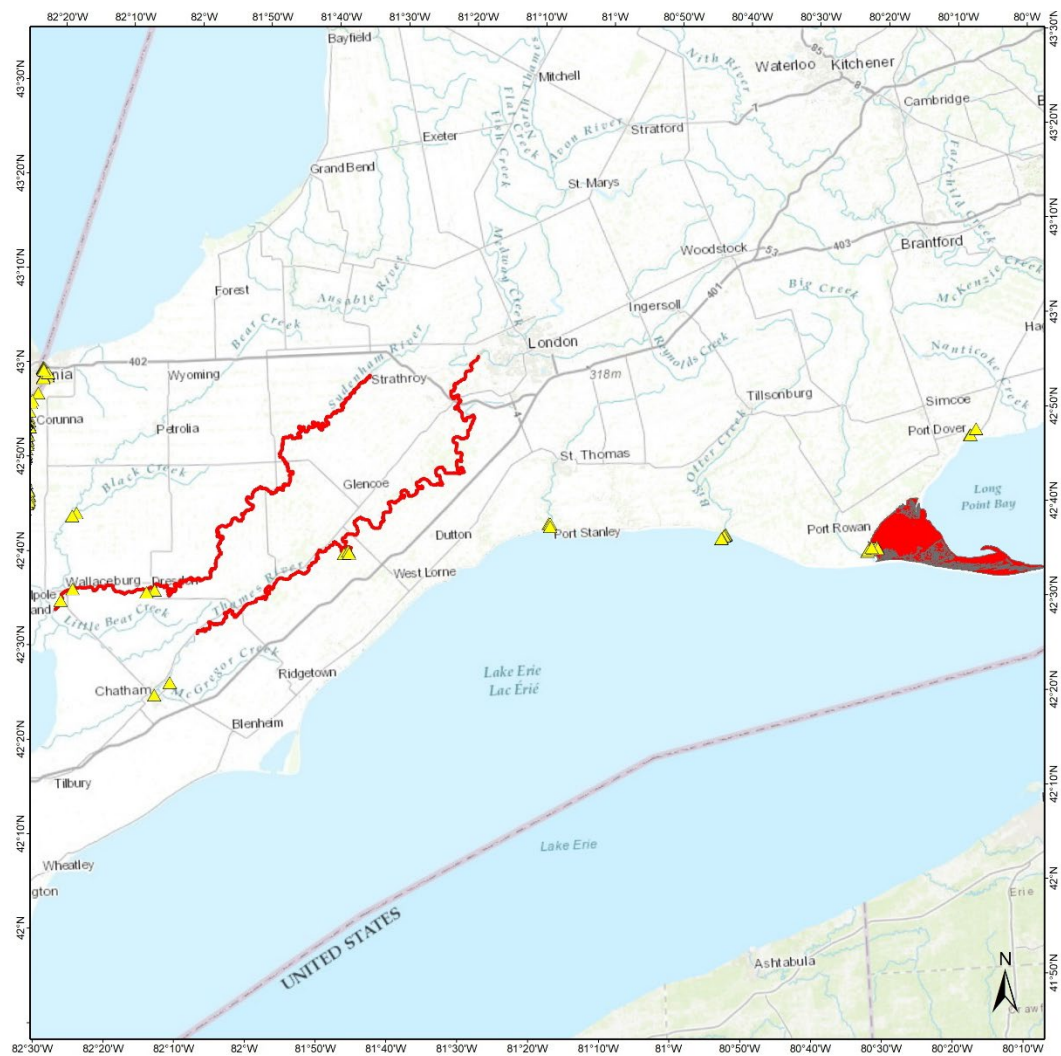


Figure A5.5. Spatial distribution of Eastern Sand Darter critical habitat (red lines and shaded area) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.

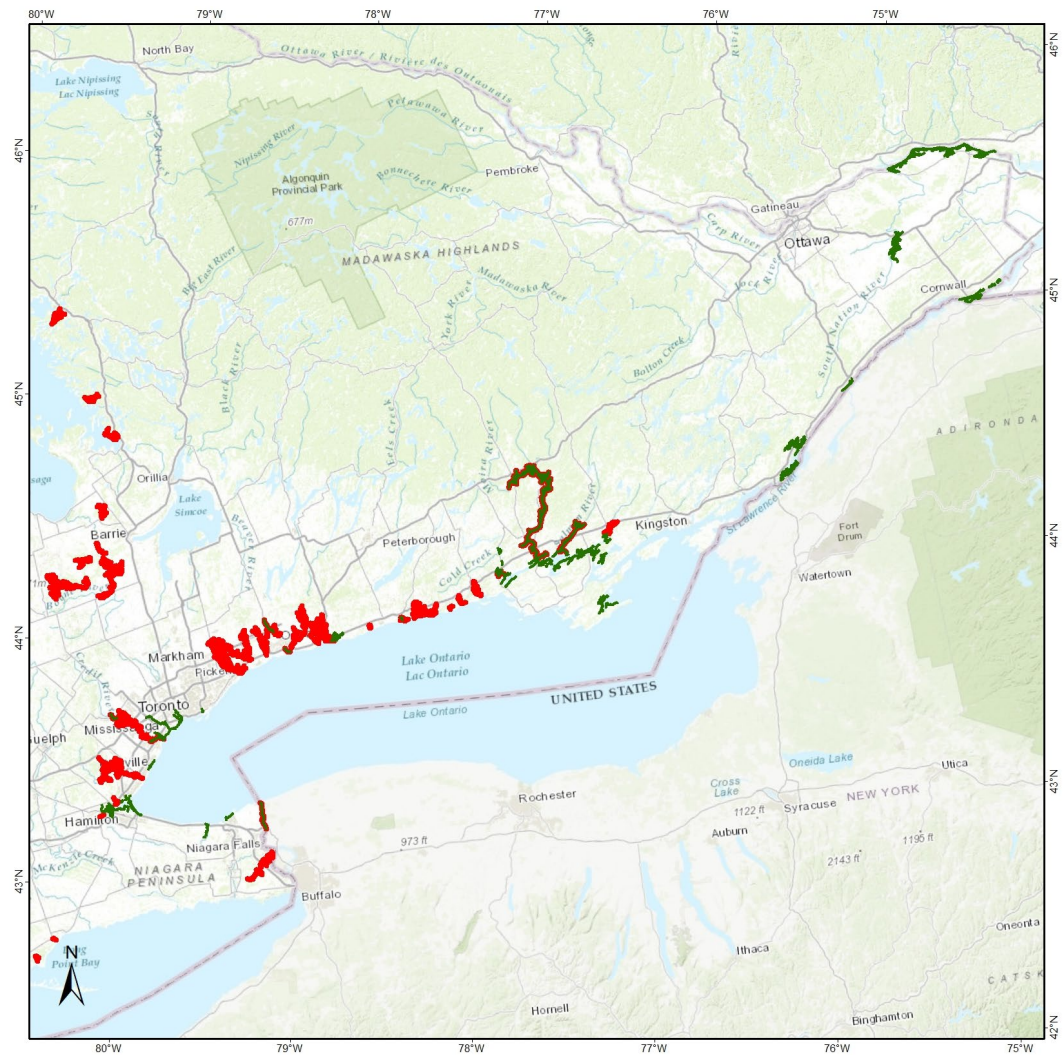


Figure A5.6. Spatial distribution of American Eel (1998–2017; green shaded area) and larval Sea Lamprey (2011–2017; red shaded area) in Ontario's Great Lakes region.



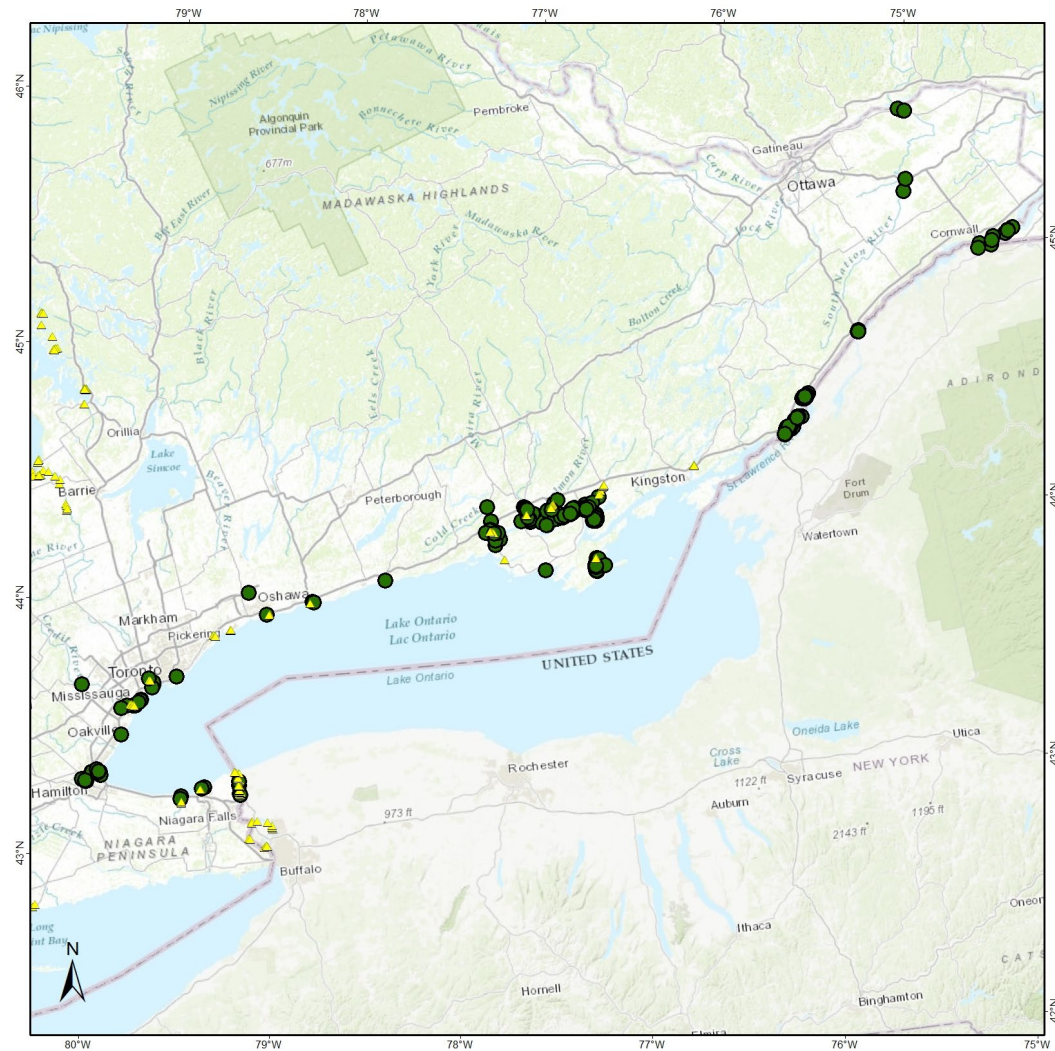


Figure A5.7. Spatial distribution of American Eel records (1998–2017; green circles) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.

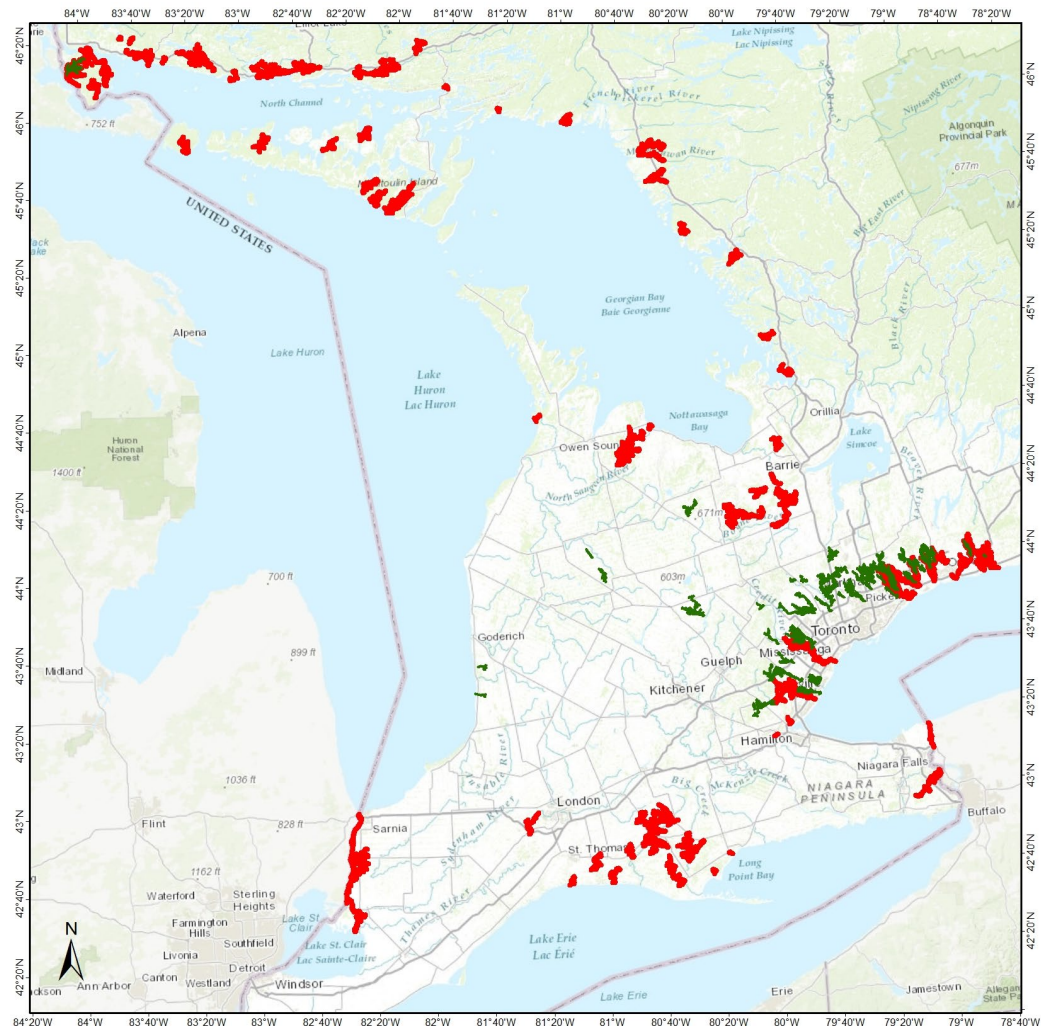


Figure A5.8. Spatial distribution of Redside Dace (1998–2017; green shaded area) and larval Sea Lamprey (2011–2017; red shaded area) in Ontario's Great Lakes region.

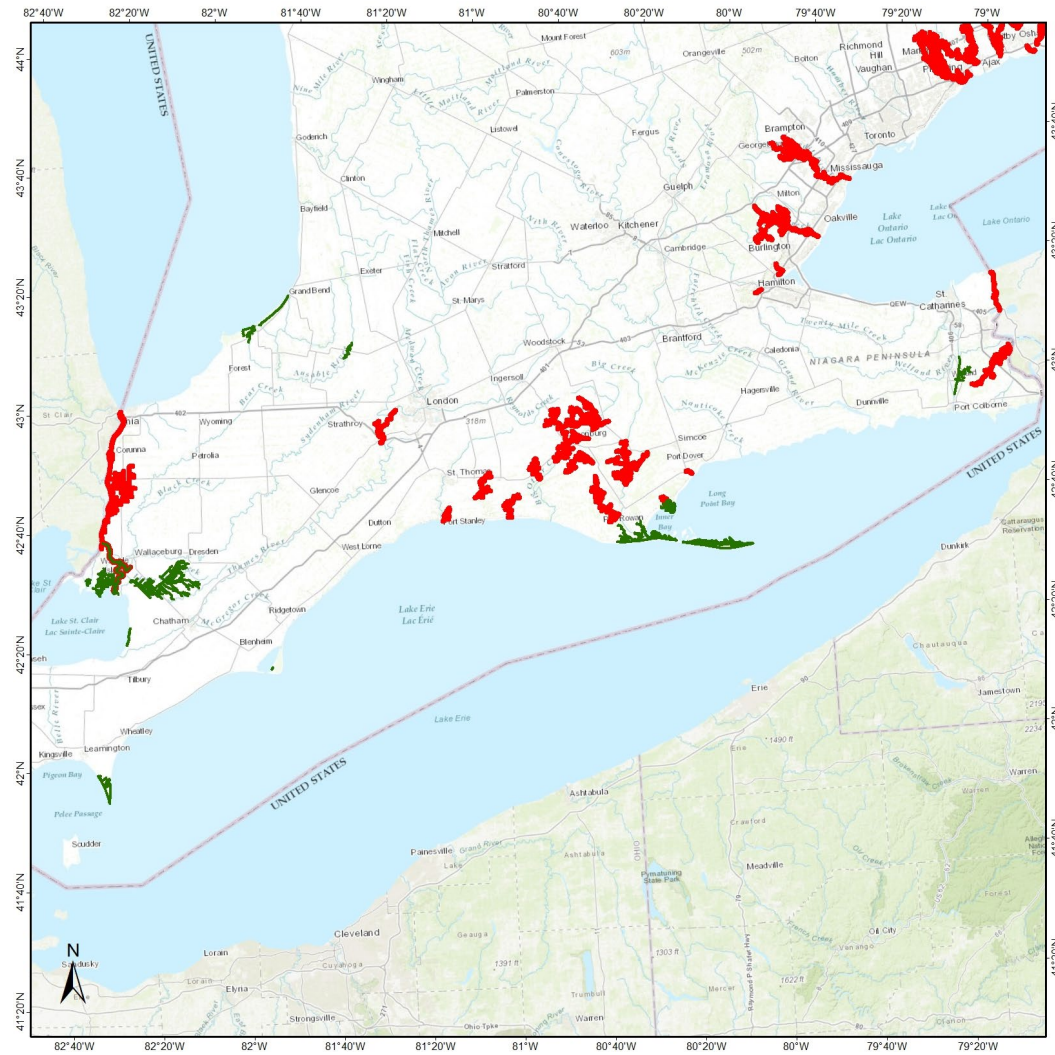


Figure A5.9. Spatial distribution of Lake Chubsucker (1998–2017; green shaded area) and larval Sea Lamprey (2011–2017; red shaded area) in Ontario's Great Lakes region.



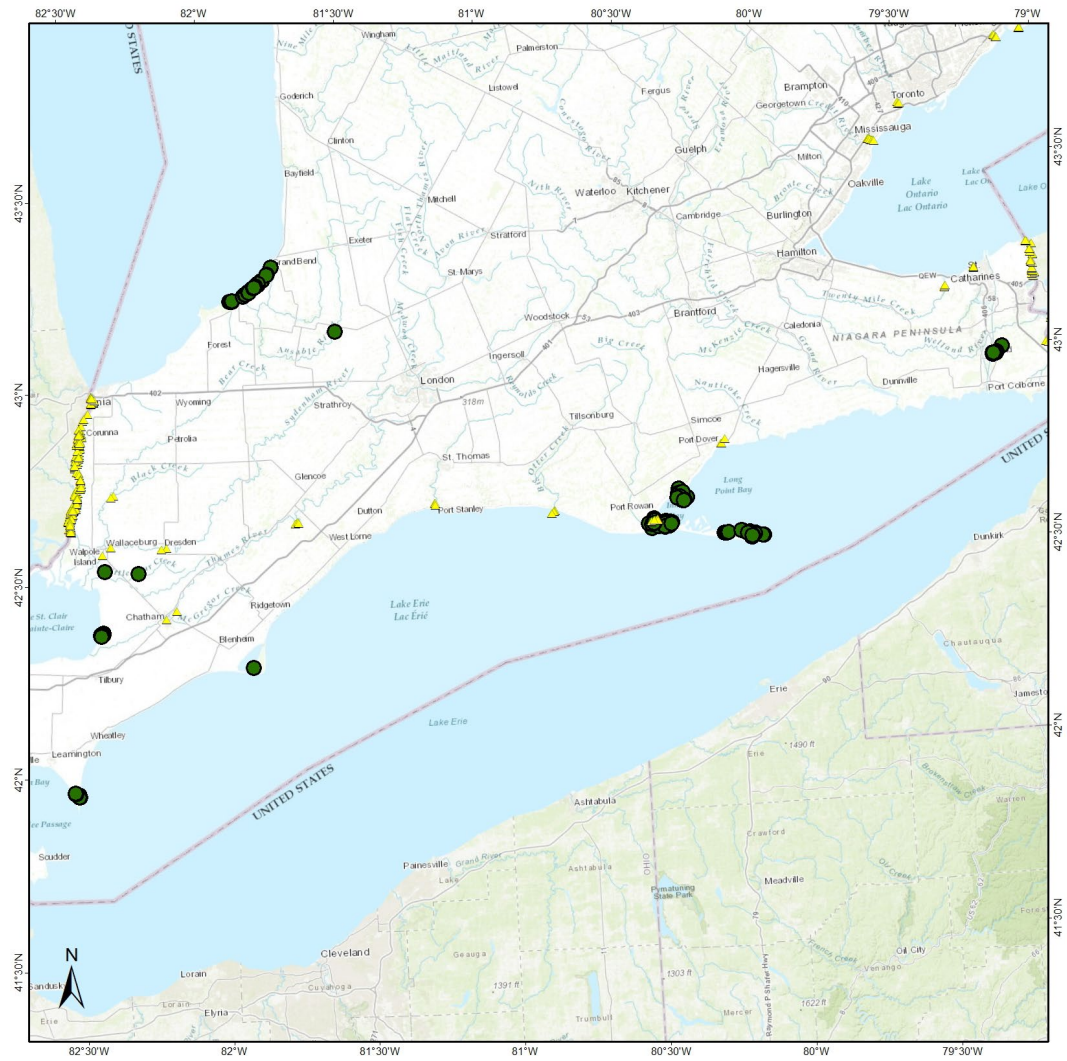


Figure A5.10. Spatial distribution of Lake Chubsucker records (1998–2017; green circles) and Bayluscide application locations (yellow triangles) in Ontario.

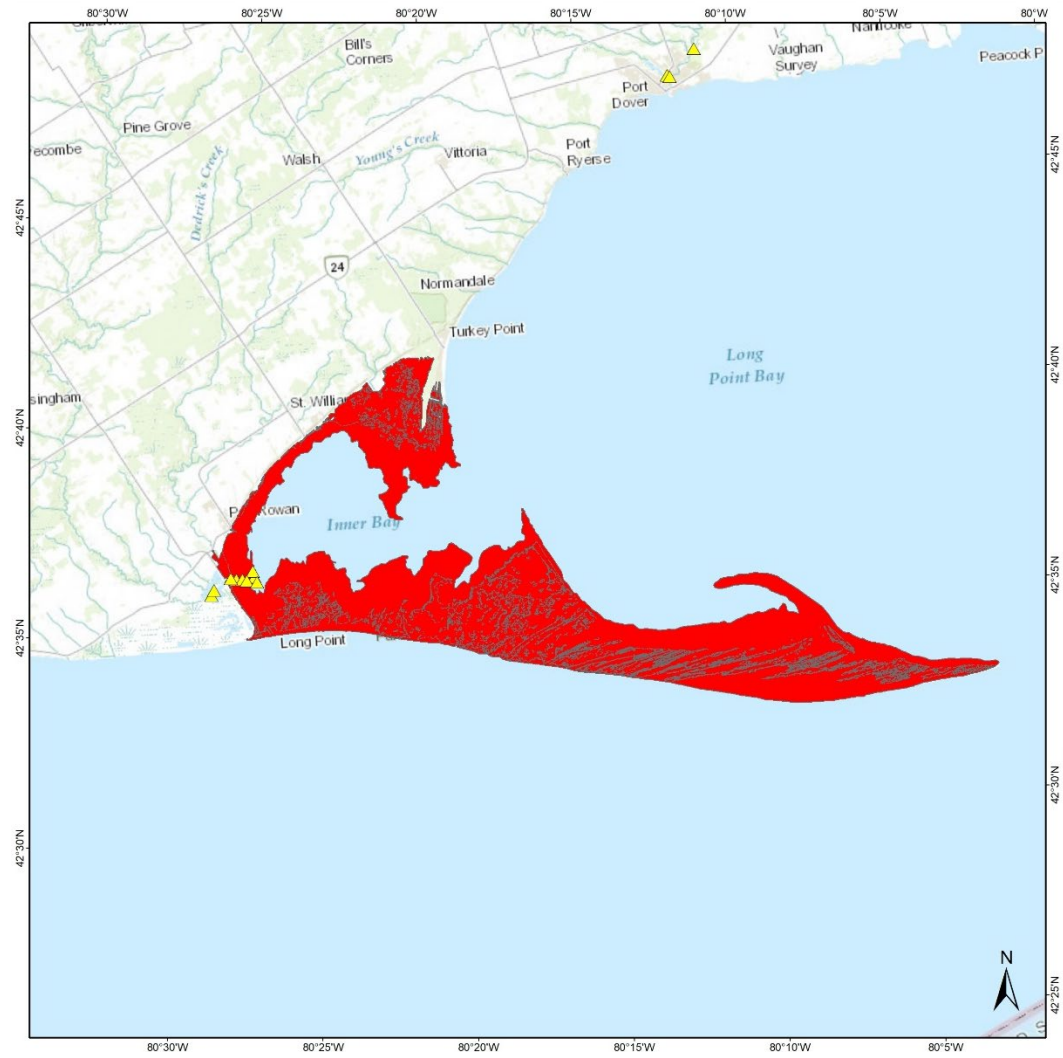


Figure A5.11. Spatial distribution of Lake Chubsubucker critical habitat (red shaded area) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.

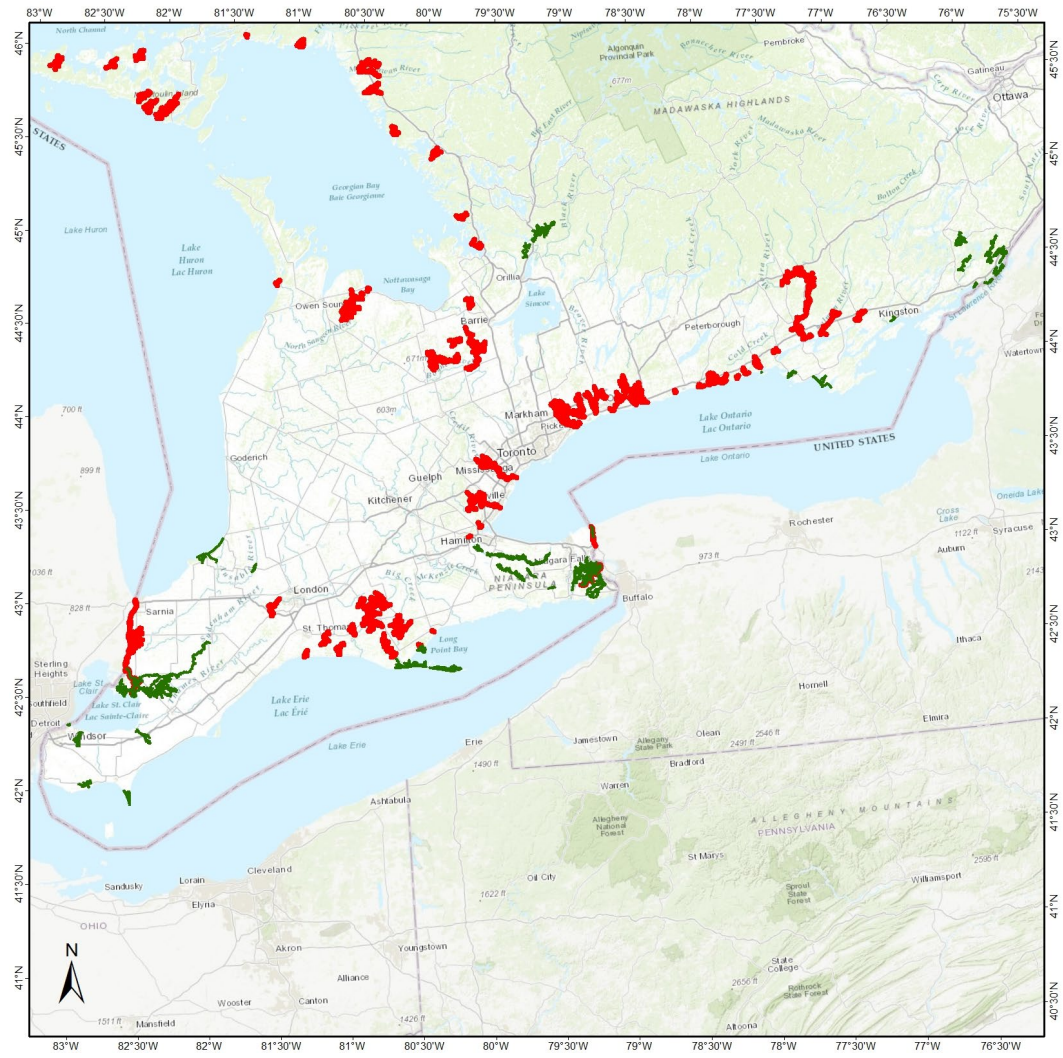


Figure A5.12. Spatial distribution of Grass Pickerel (1998–2017; green shaded area) and larval Sea Lamprey (2011–2017; red shaded area) in Ontario’s Great Lakes region.



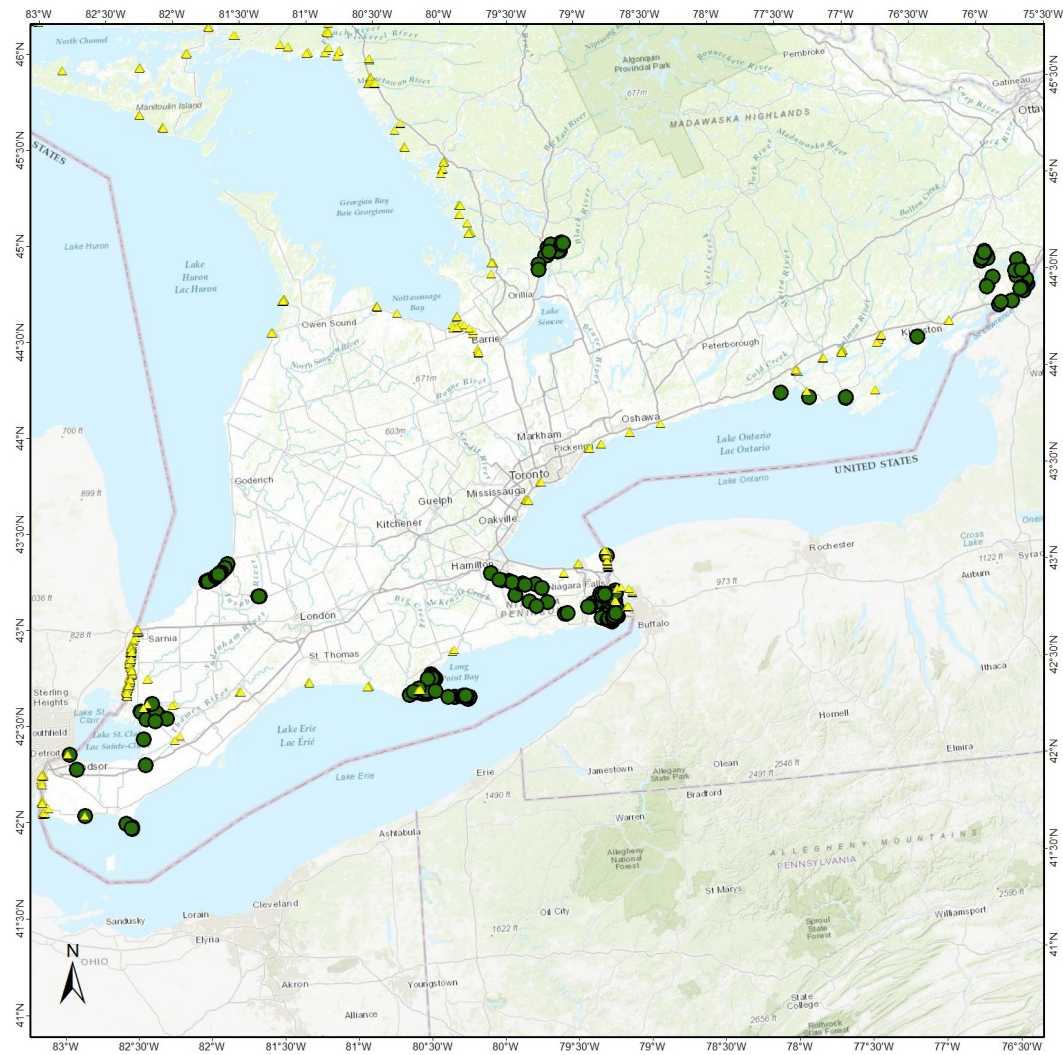


Figure A5.13. Spatial distribution of Grass Pickerel records (1998–2017; green circles) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.

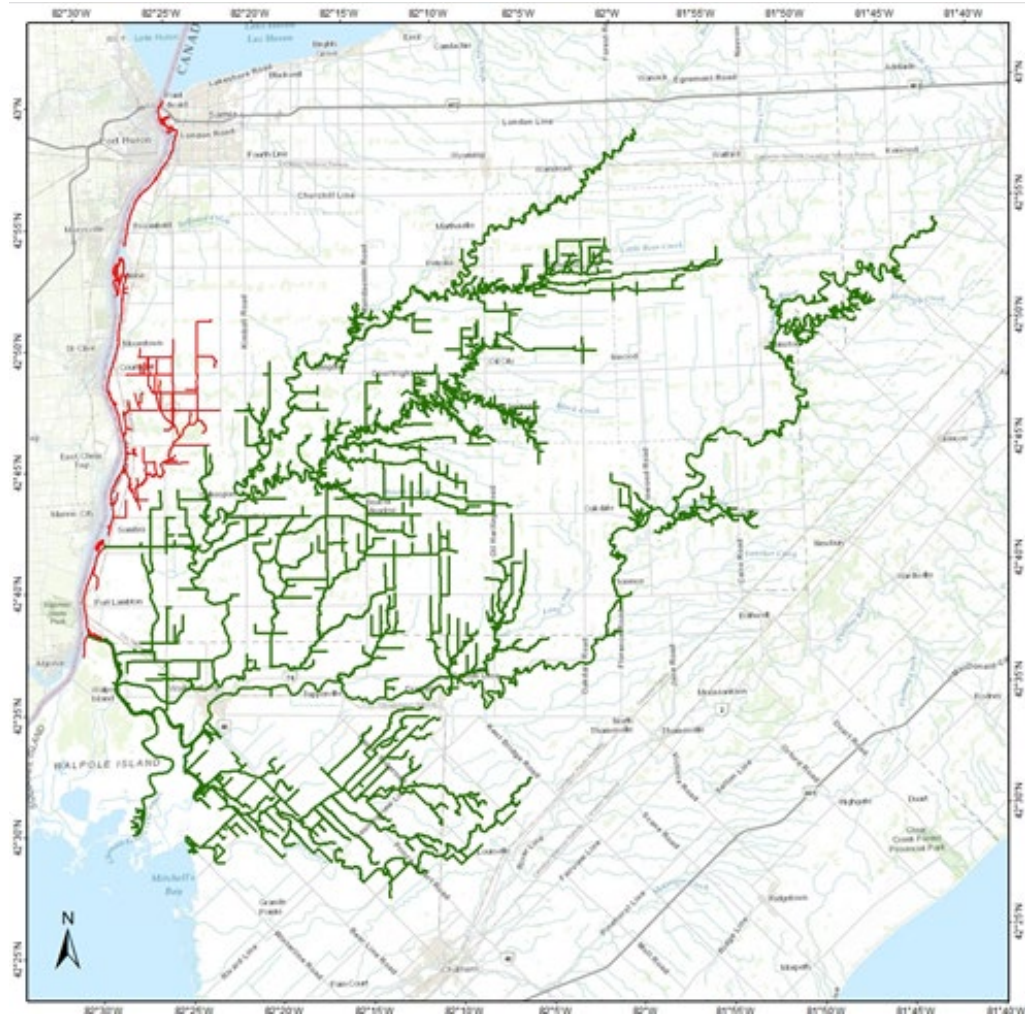


Figure A5.14. Spatial distribution of Blackstripe Topminnow (1998–2017, green lines) and larval Sea Lamprey (2011–2017; red lines) in Ontario's Great Lakes region.



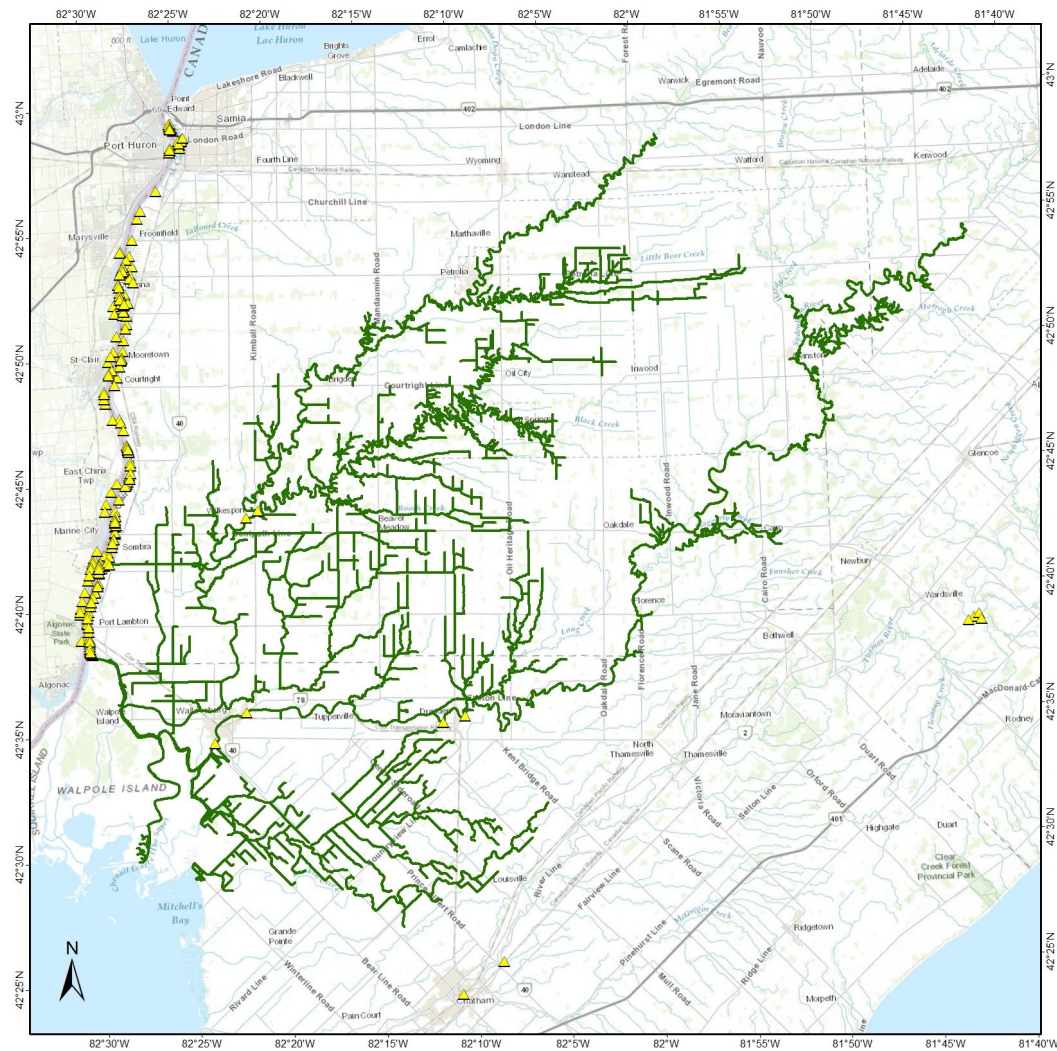


Figure A5.15. Spatial distribution of Blackstripe Topminnow (1998–2017; green lines) and Baylucside application locations (2011–2017; yellow triangles) in Ontario's Great Lakes region.

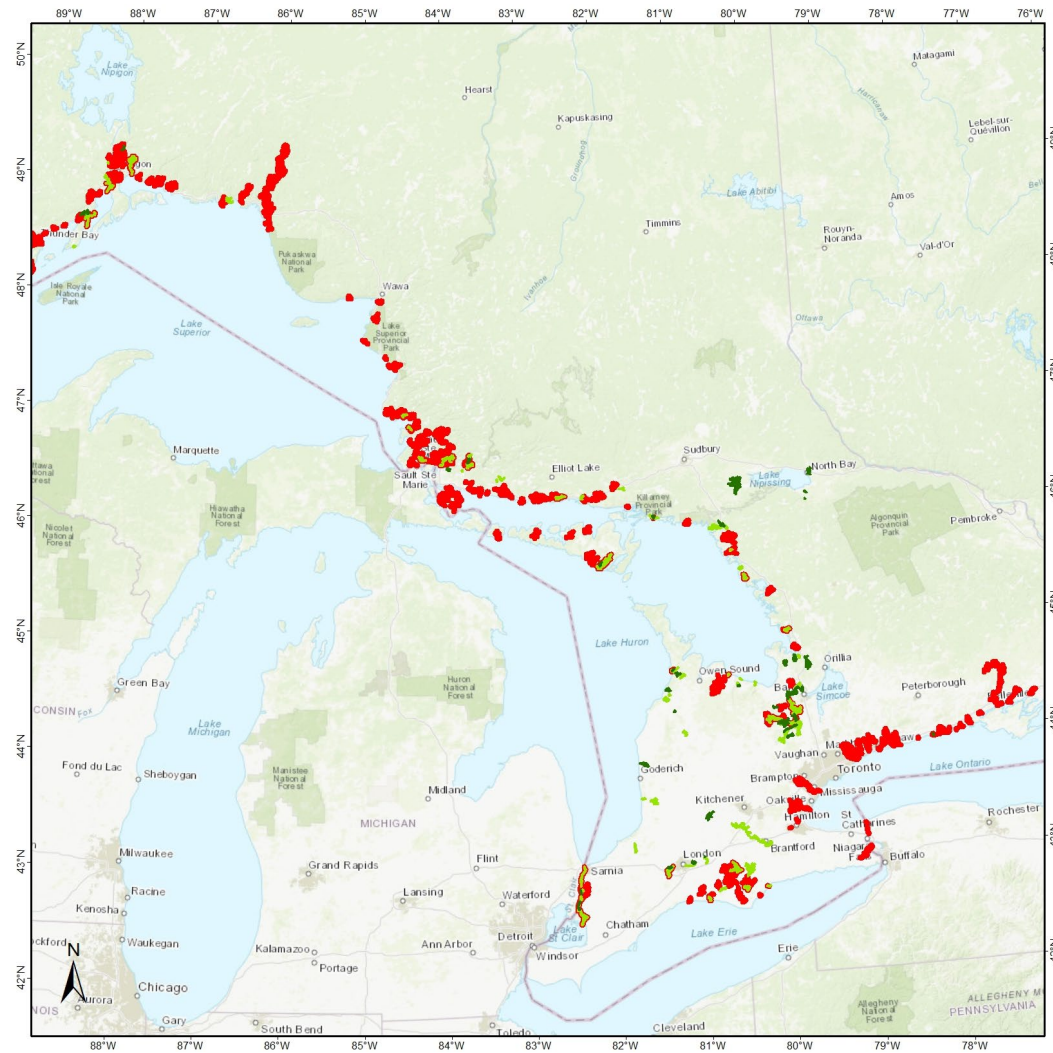


Figure A5.16. Spatial distribution of Northern Brook Lamprey (1998–2017; green shaded area), *Ichthyomyzon* sp. (light green shaded area), and larval Sea Lamprey (2011–2017; red shaded area) in Ontario's Great Lakes region.

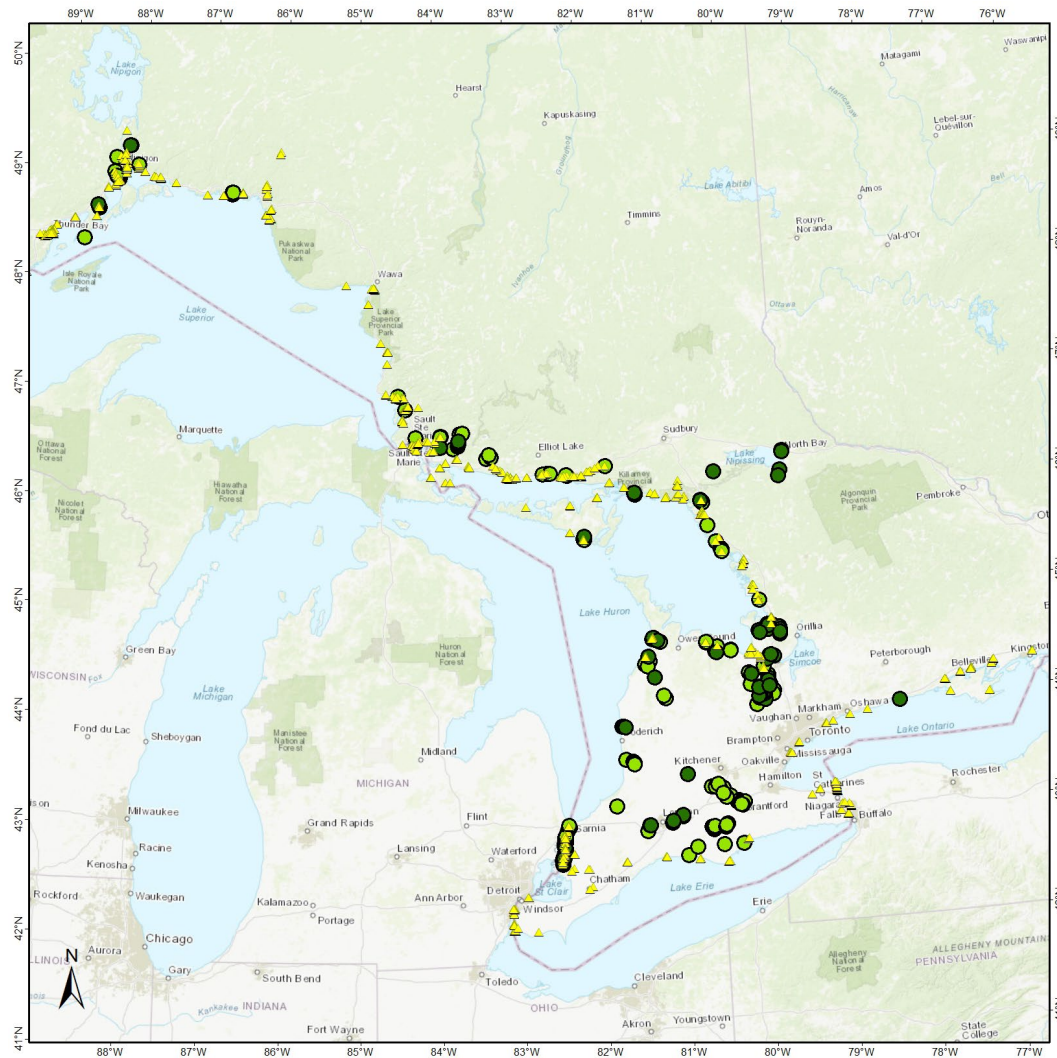


Figure A5.17. Spatial distribution of Northern Brook Lamprey records (1998–2017; green circles), *Ichthyomyzon* sp. records (1998–2017; light green circles), and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.



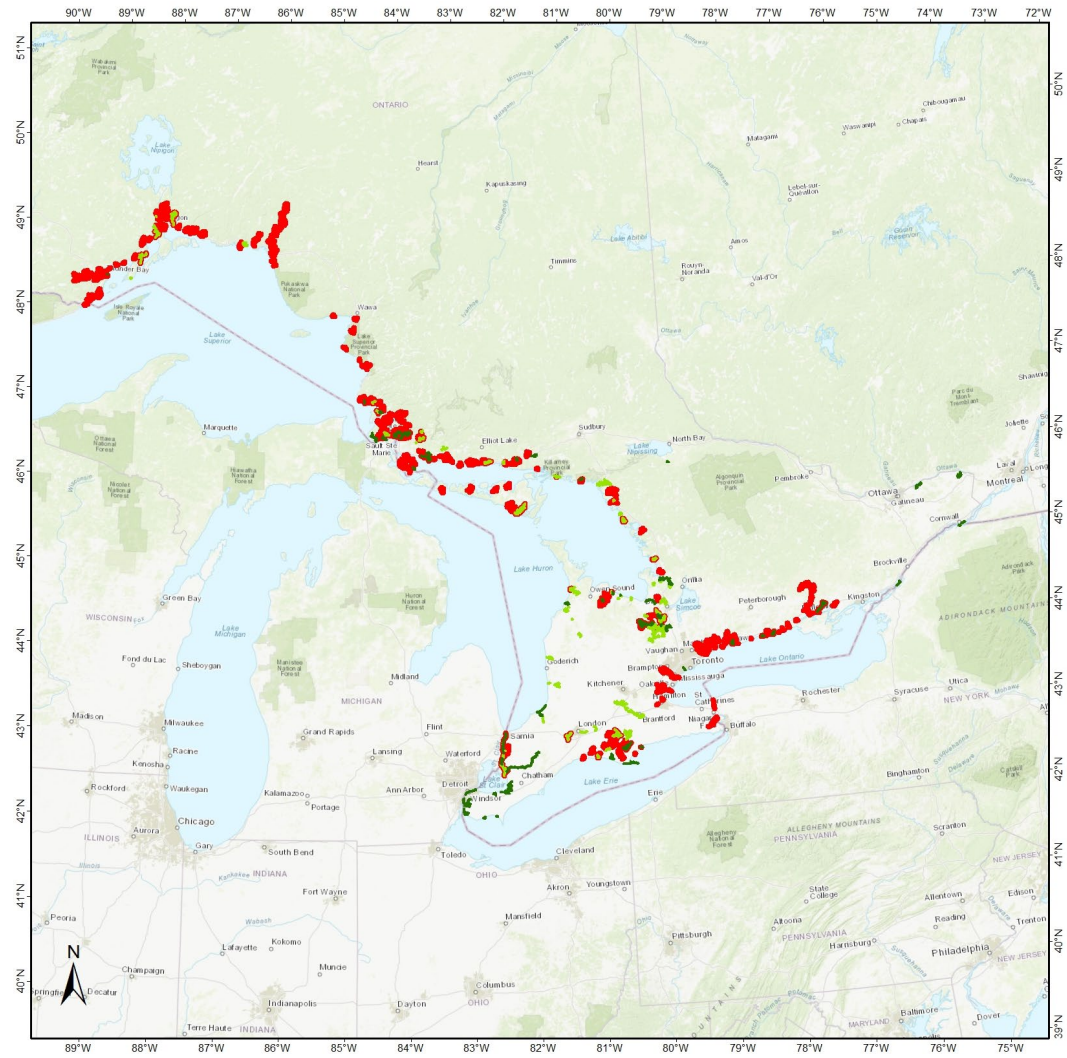


Figure A5.18. Spatial distribution of Silver Lamprey (1998–2017; green shaded area), Ichthyomyzon sp. (1998–2017; light green shaded area), and larval Sea Lamprey (2011–2017; red shaded area) in Ontario's Great Lakes region.

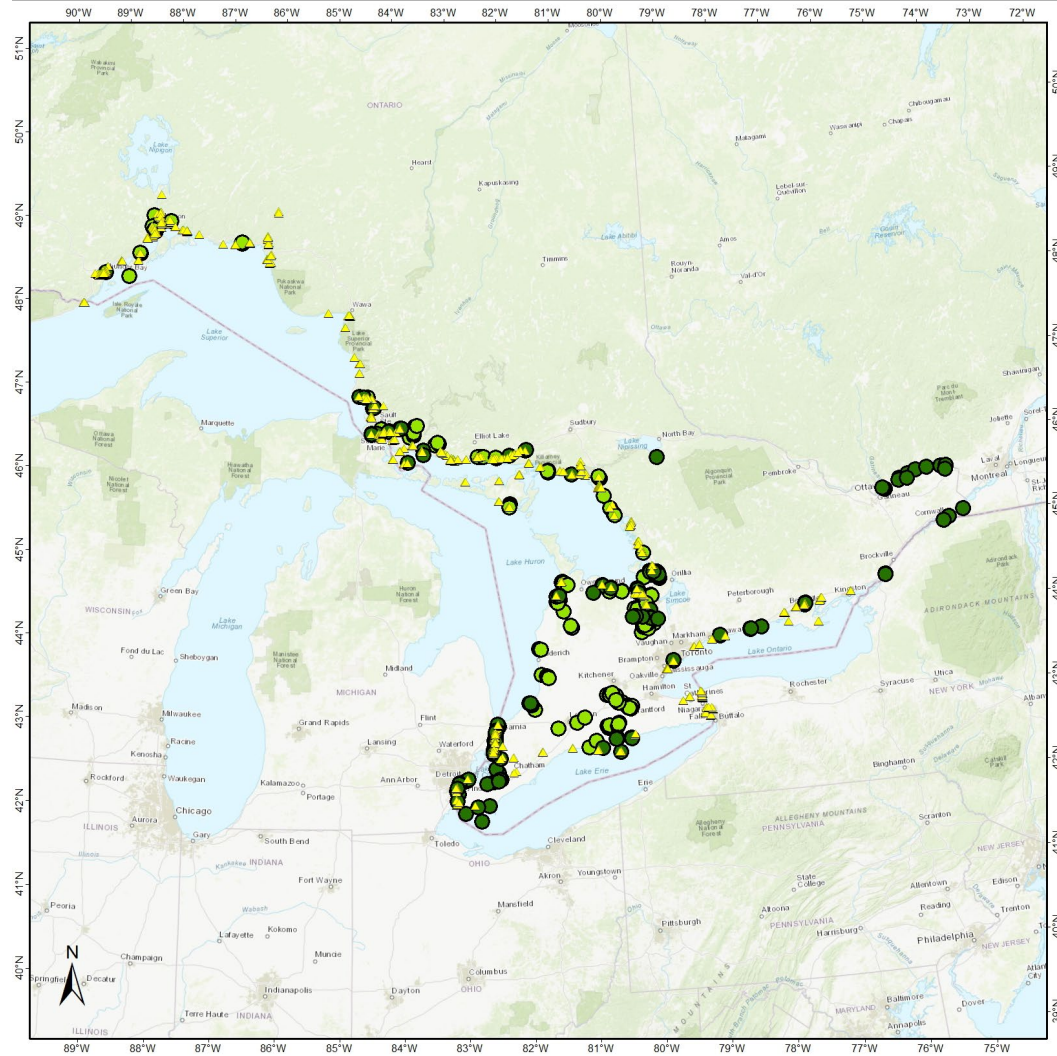


Figure A5.19. Spatial distribution of Silver Lamprey (1998–2017; green circles), Ichthyomyzon sp. (1998–2017; light green circles), and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.

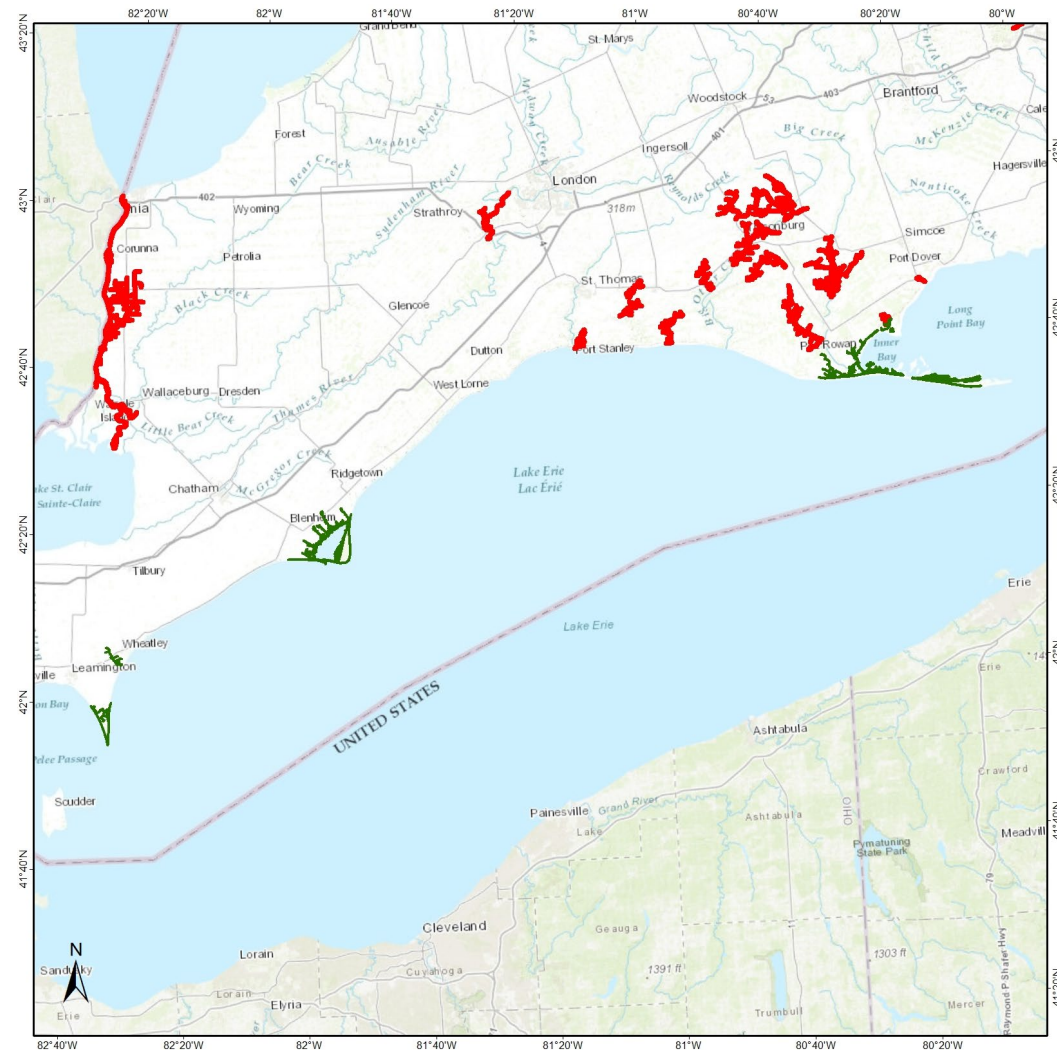


Figure A5.20. Spatial distribution of Spotted Gar (1998–2017; green shaded area) and larval Sea Lamprey (2011–2017; red shaded area) in Ontario’s Great Lakes region.



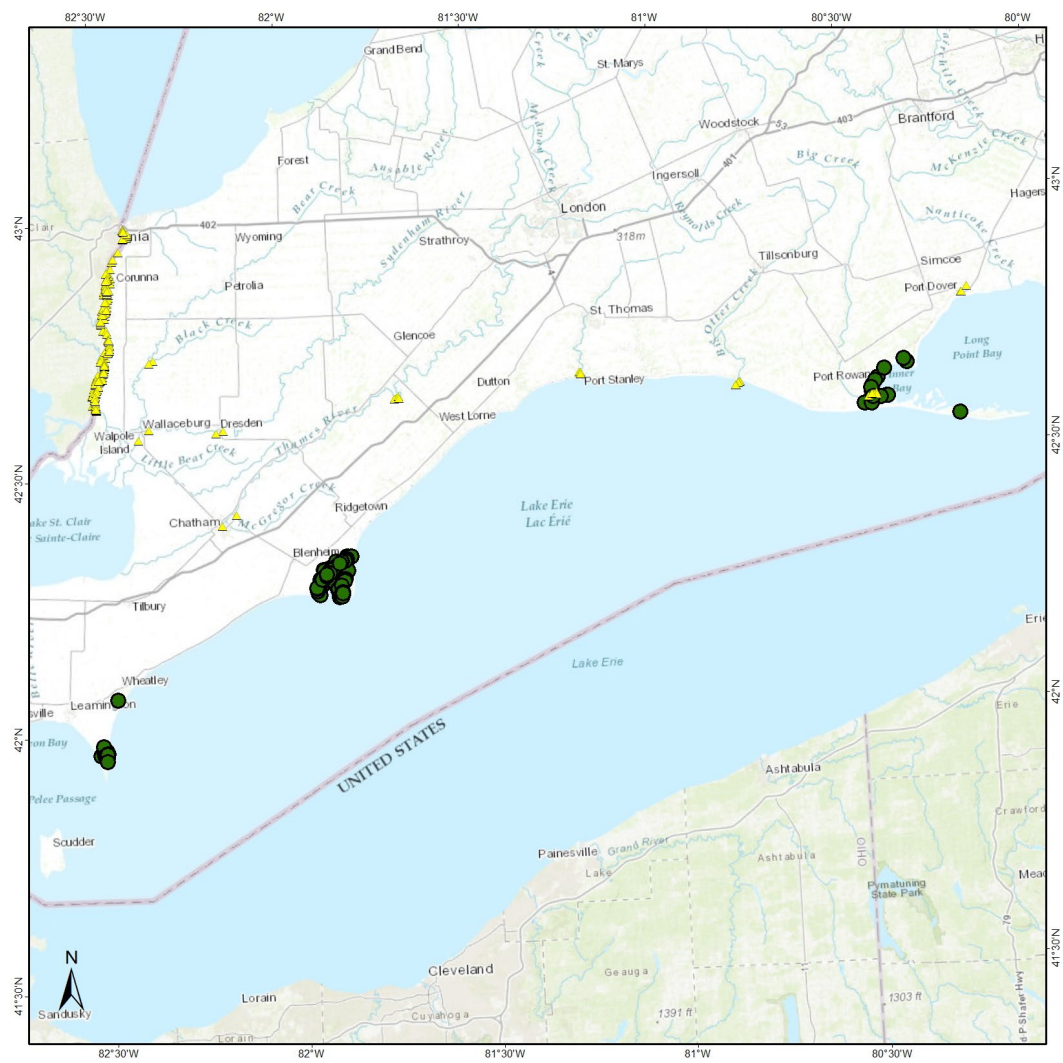


Figure A5.21. Spatial distribution of Spotted Gar records (1998–2017; green circles) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.

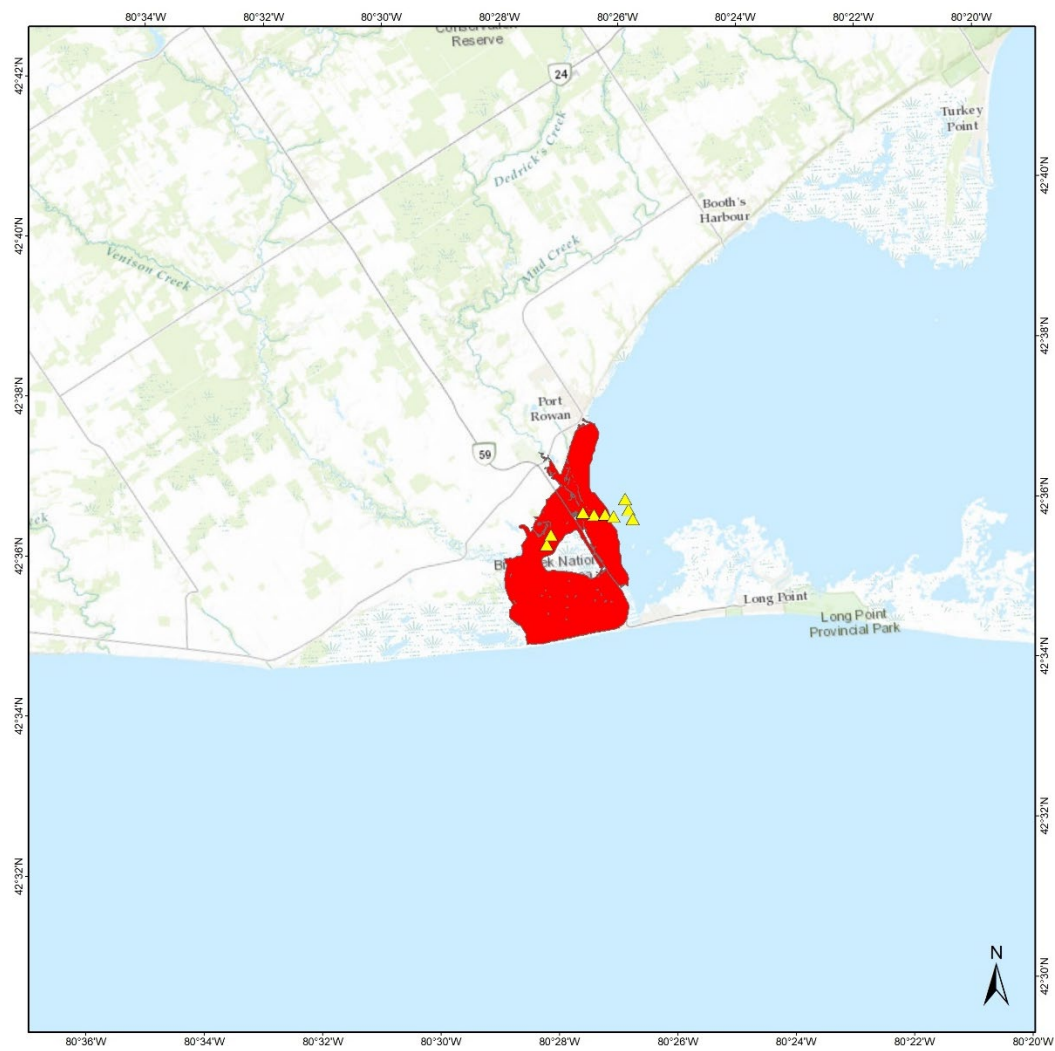


Figure 10. Spatial distribution of Spotted Gar critical habitat (red shaded area) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.





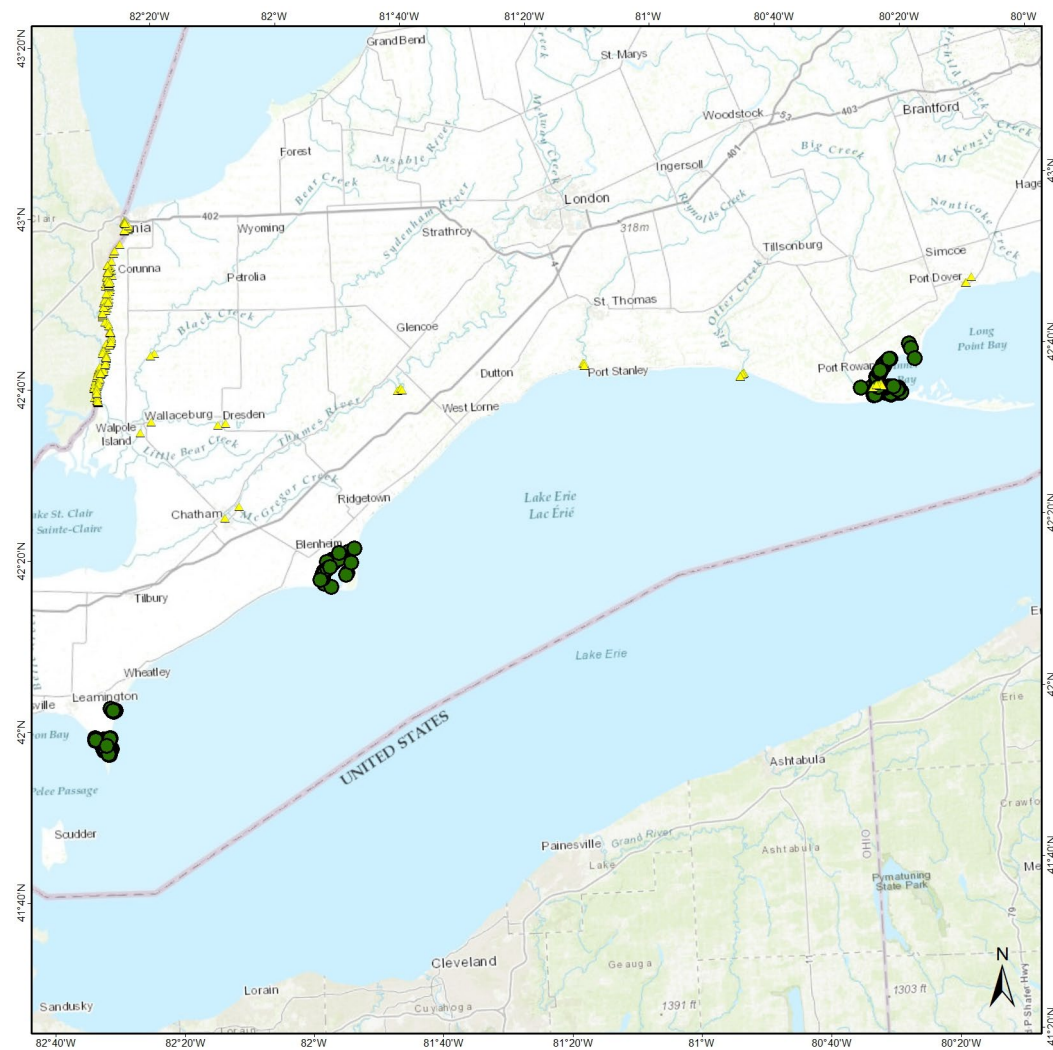


Figure A5.24. Spatial distribution of Warmouth records (1998–2017; green circles) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.

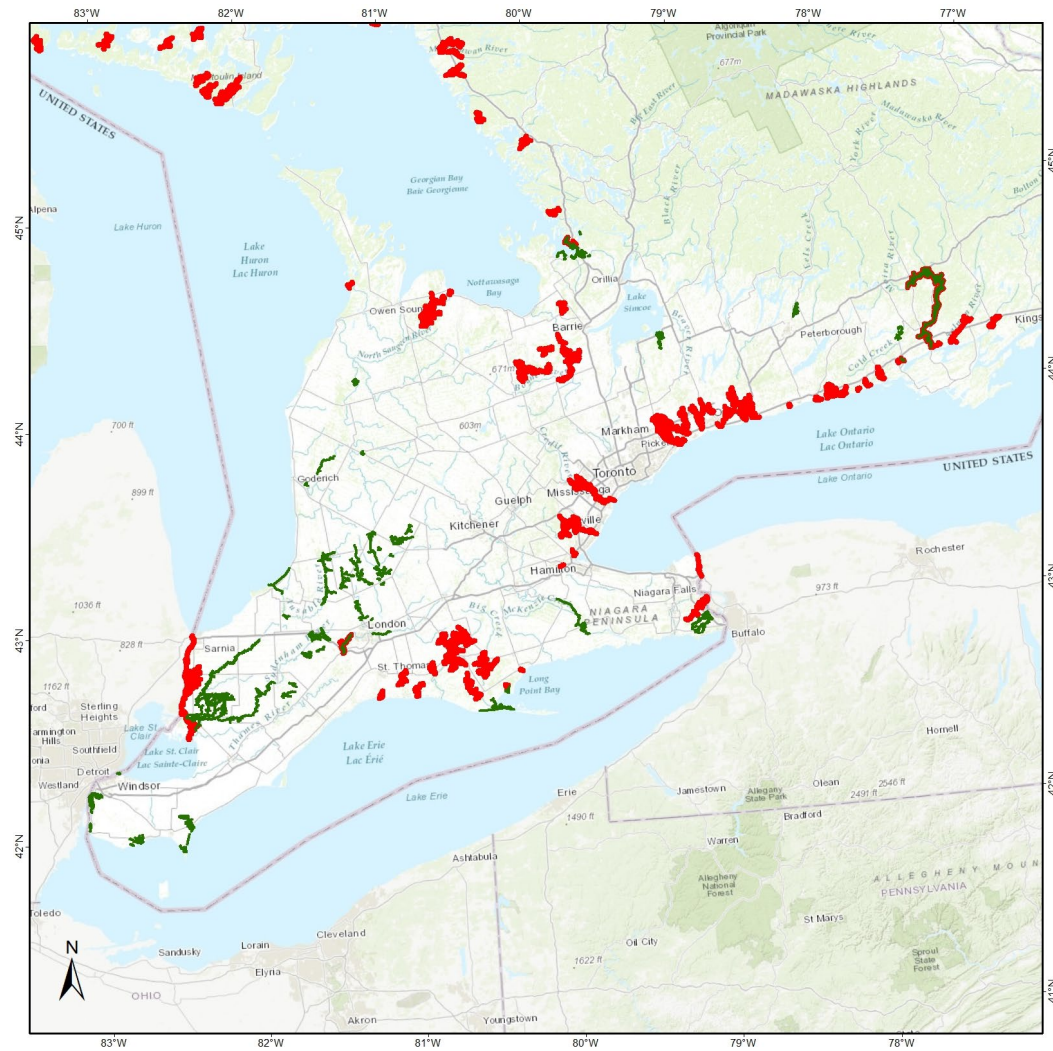


Figure A5.25. Spatial distribution of Northern Sunfish (1998–2017; green shaded area) and larval Sea Lamprey (2011–2017; red shaded area) in Ontario’s Great Lakes region.



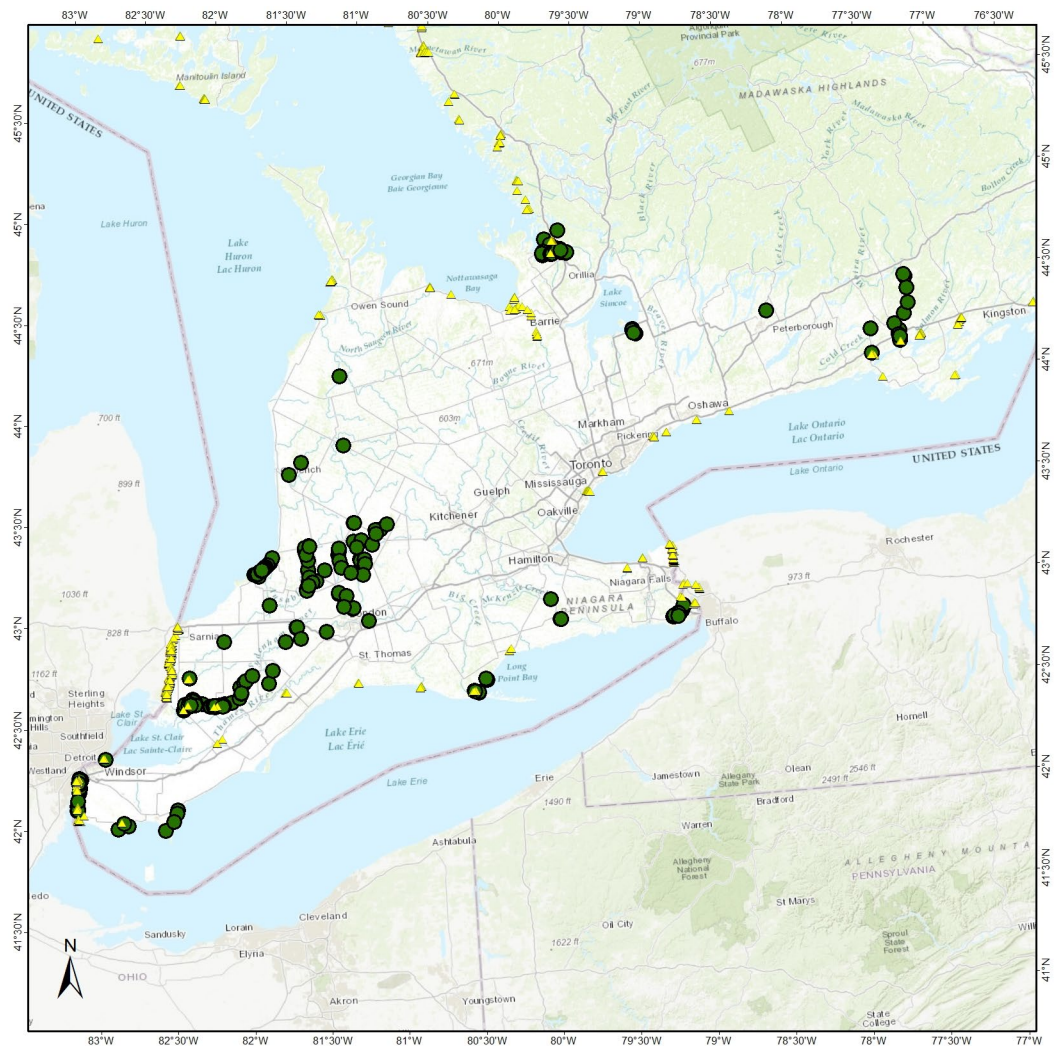


Figure A5.26. Spatial distribution of Northern Sunfish records (1998–2017; green circles) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.

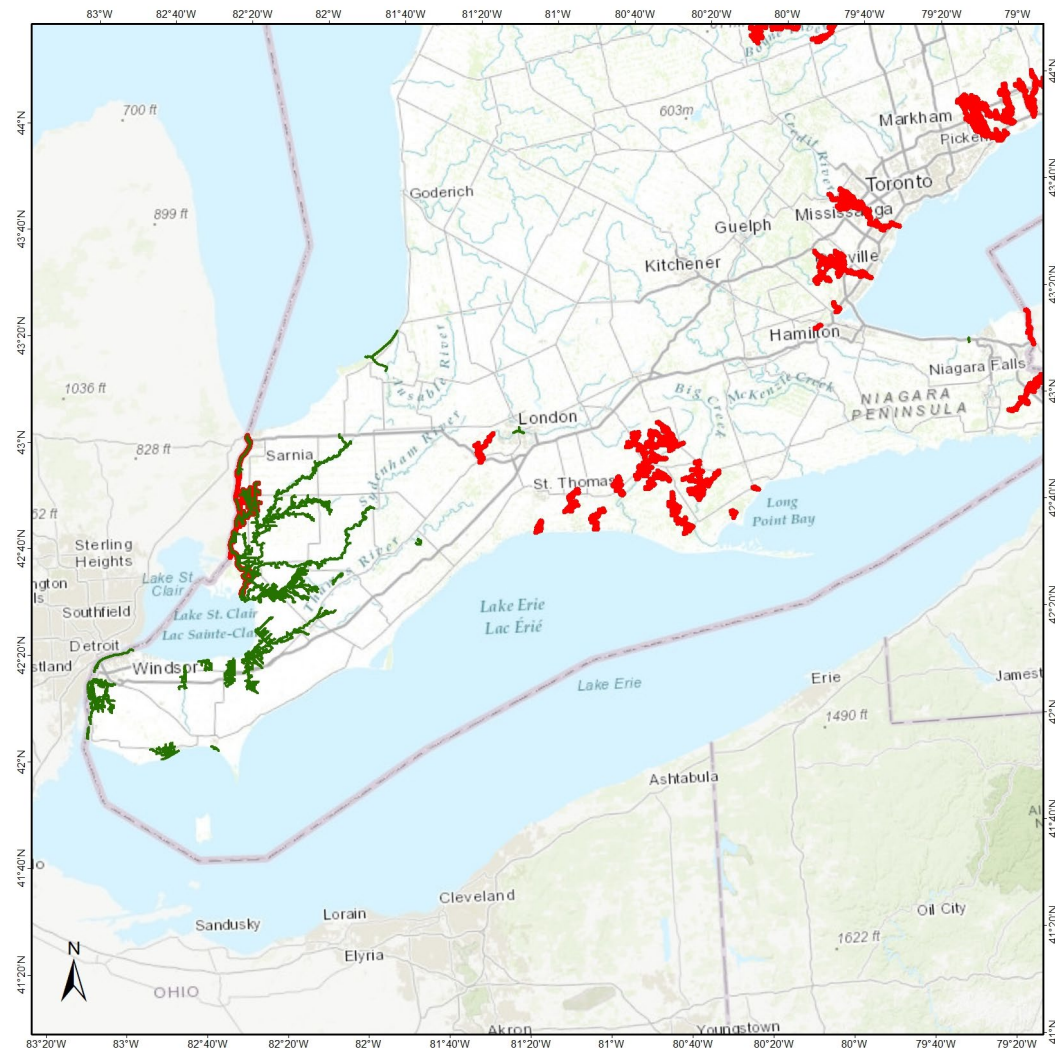


Figure A5.27. Spatial distribution of Spotted Sucker (1998–2017; green shaded area) and larval Sea Lamprey (2011–2017; red shaded area) in Ontario's Great Lakes region.

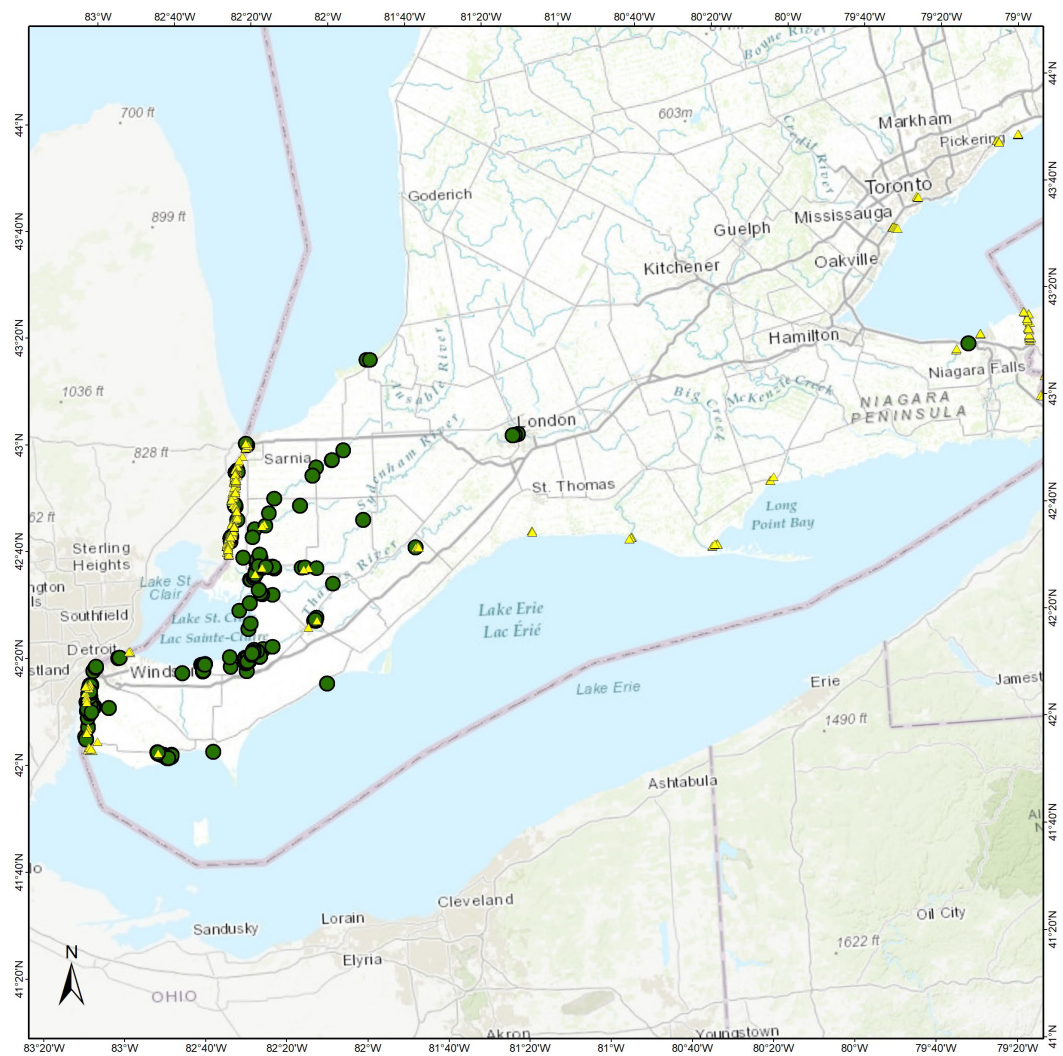


Figure A5.28. Spatial distribution of Spotted Sucker records (1998–2017; green circles) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.



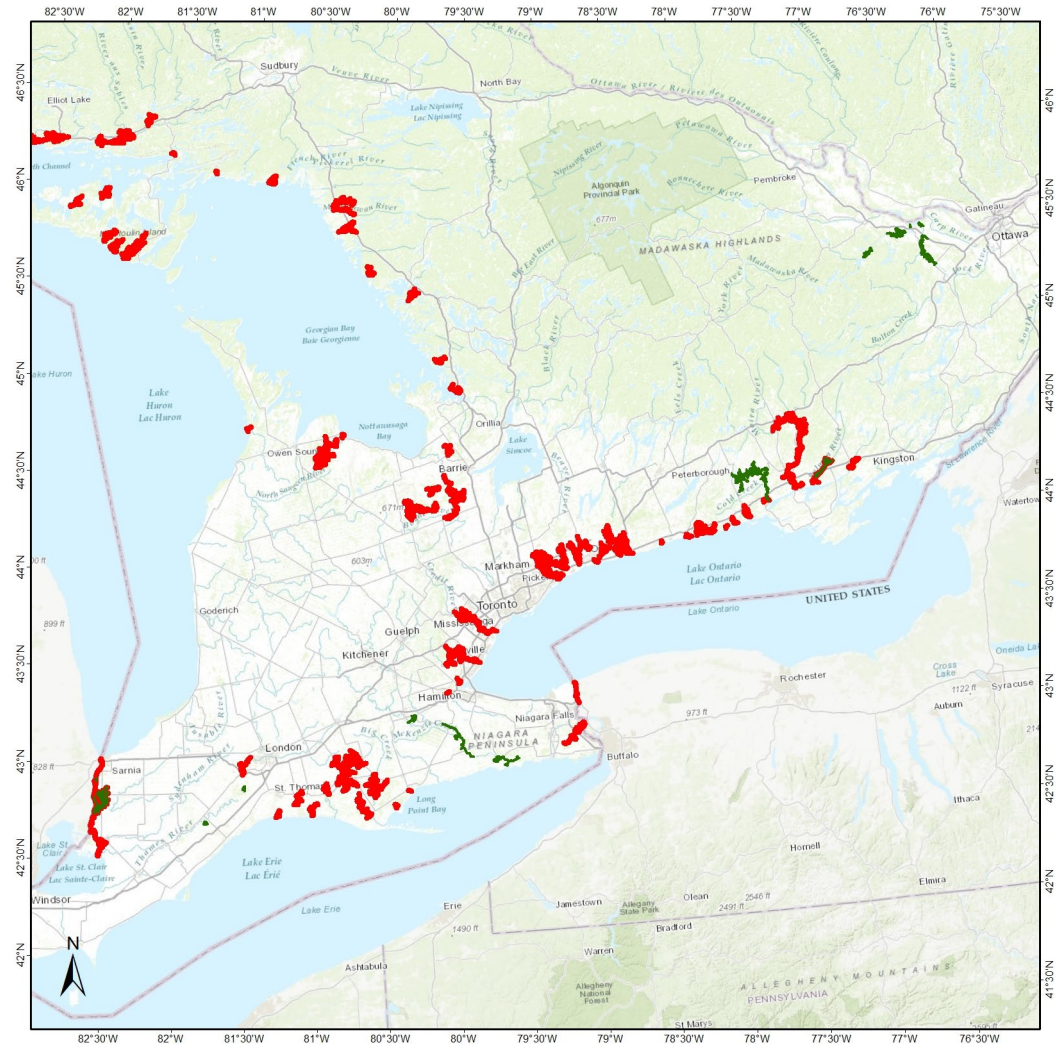


Figure A5.29. Spatial distribution of River Redhorse (1998–2017; green shaded area) and larval Sea Lamprey (2011–2017; red shaded area) in Ontario’s Great Lakes region.

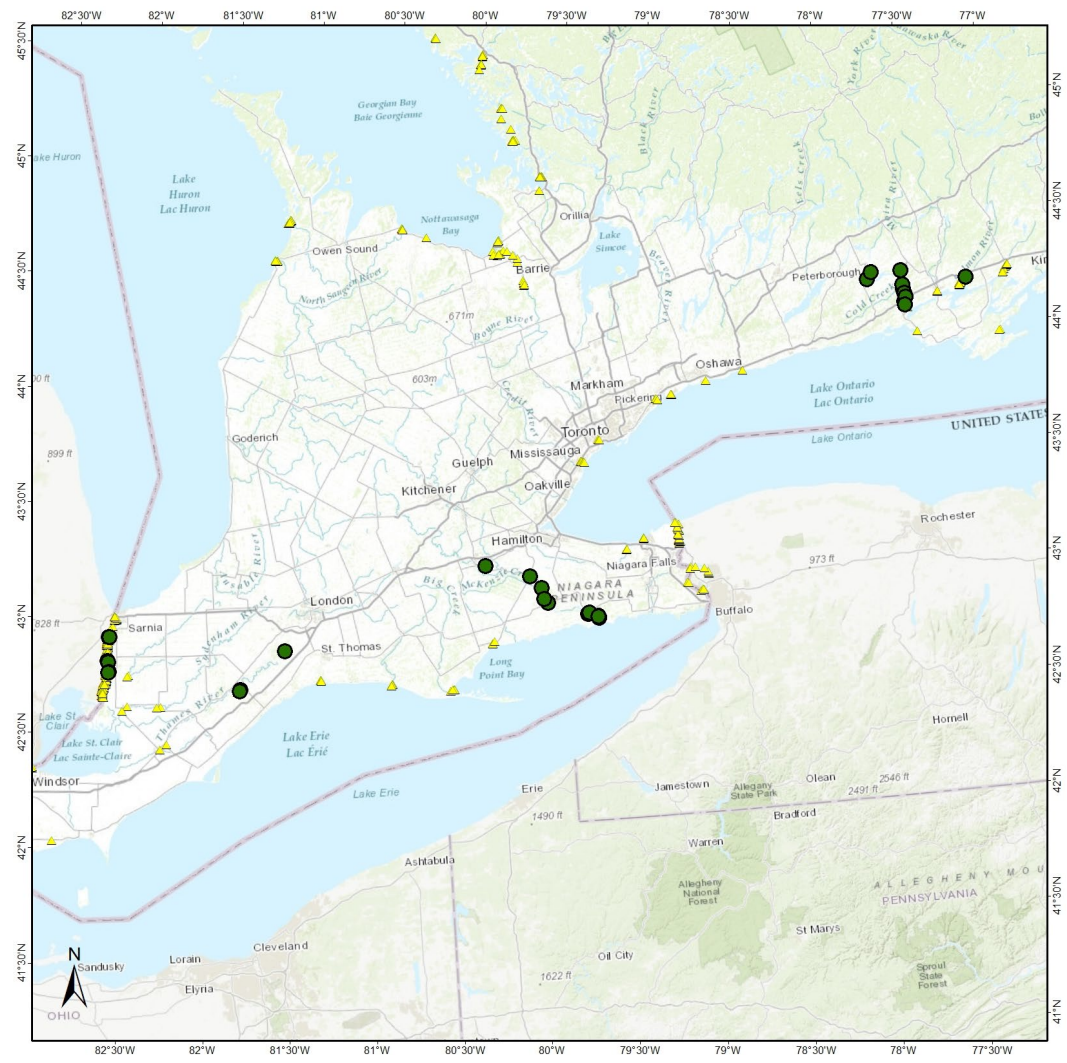


Figure A5.30. Spatial distribution of River Redhorse records (1998–2017; green circles) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.



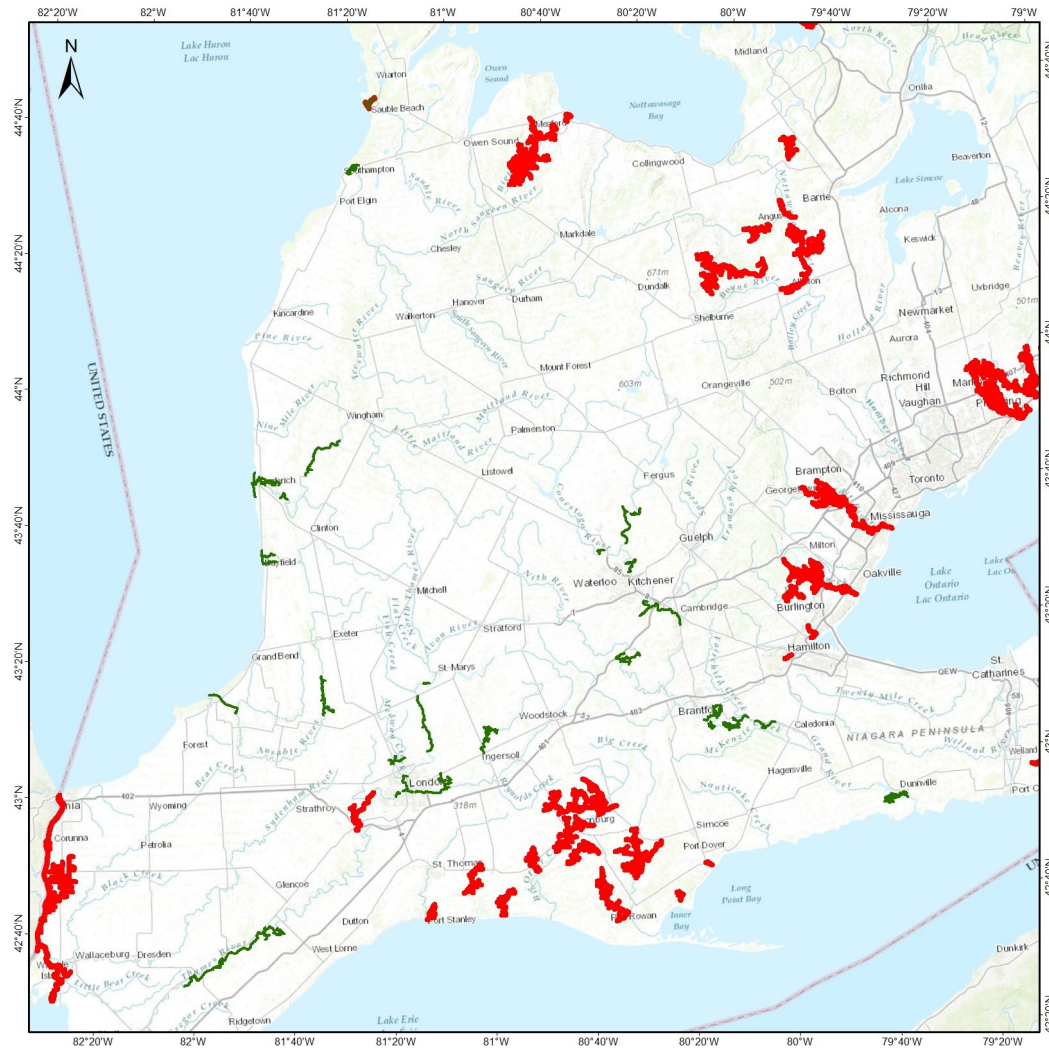


Figure A5.31. Spatial distribution of Black Redhorse (1998–2017; green shaded area) and larval Sea Lamprey (2011–2017; red shaded area) in Ontario's Great Lakes region

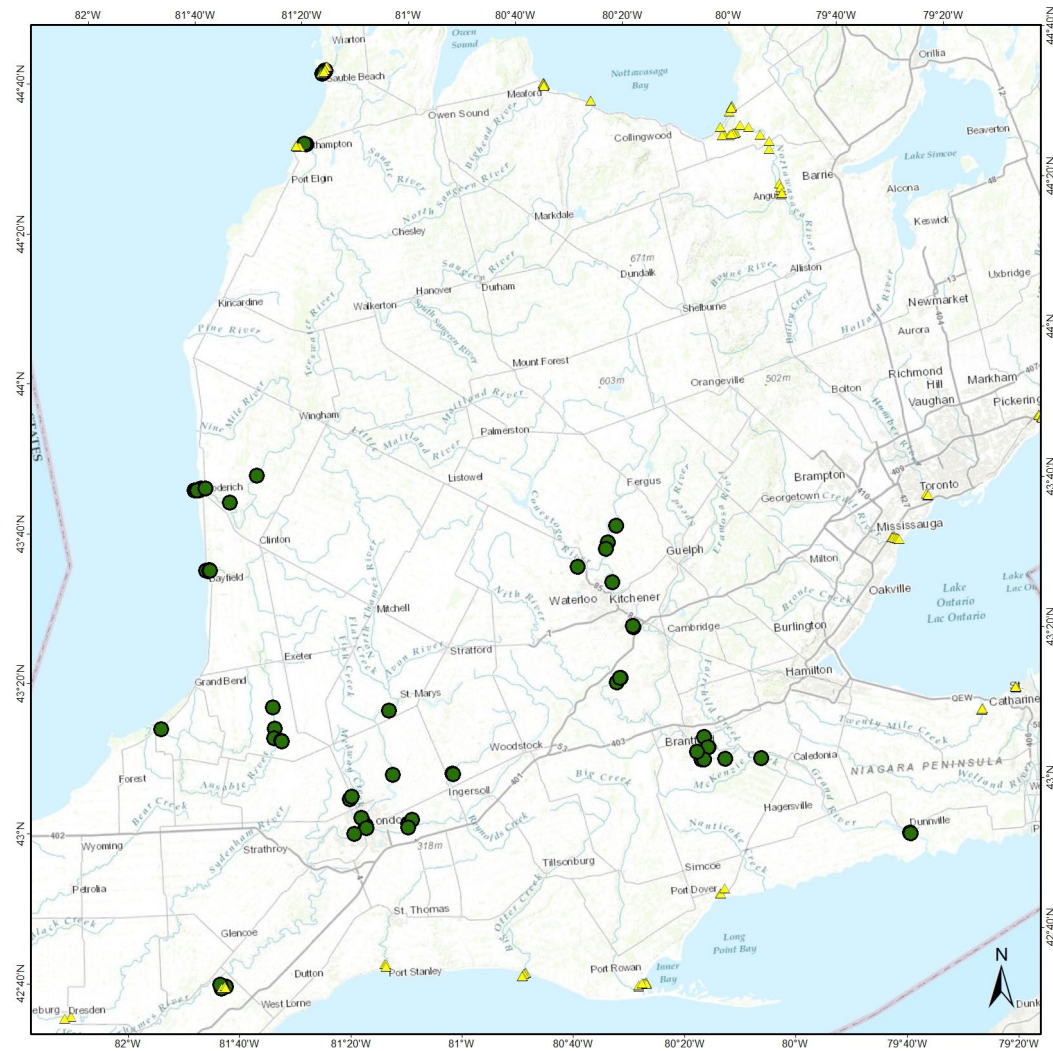


Figure A5.32. Spatial distribution of Black Redhorse records (1998–2017; green circles) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.

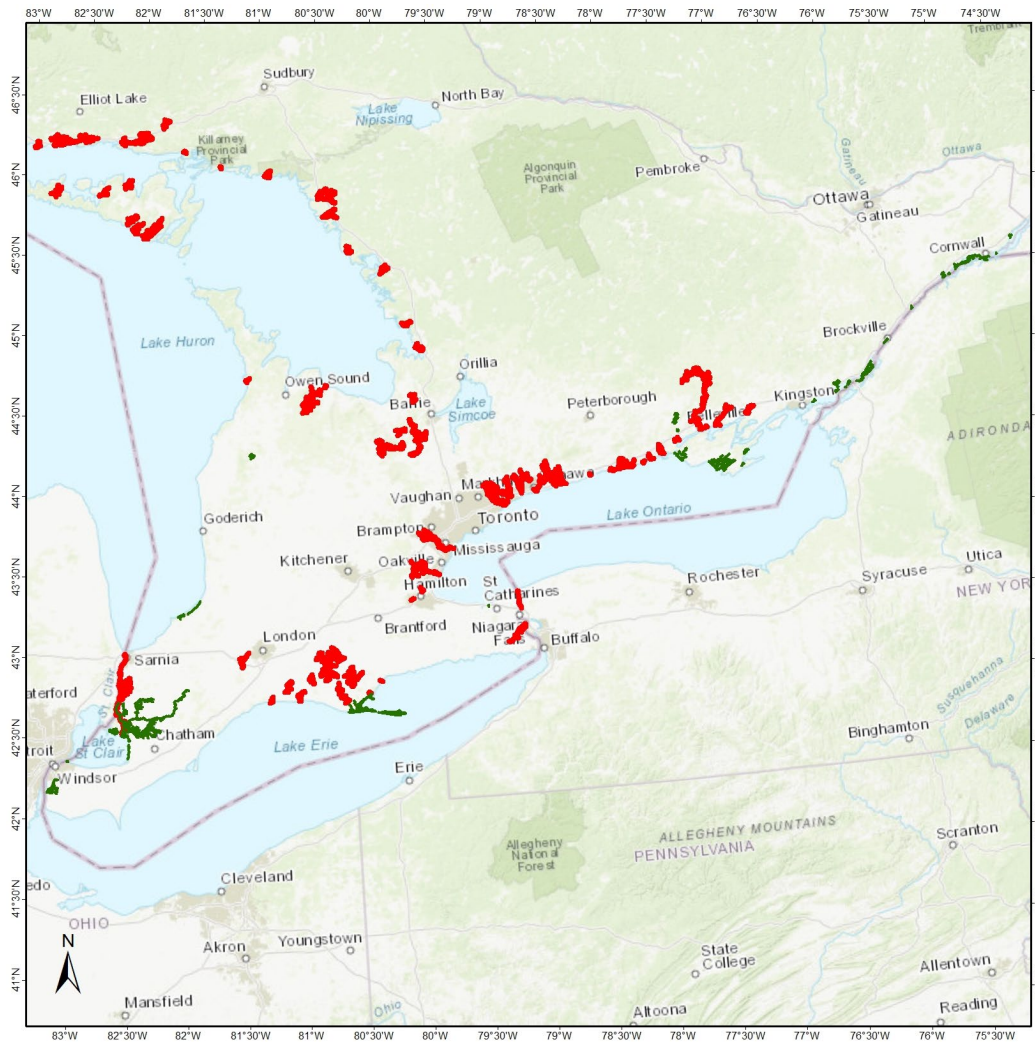


Figure A5.33. Spatial distribution of Pugnose Shiner (1998–2017; green shaded area) and larval Sea Lamprey (2011–2017; red shaded area) in Ontario's Great Lakes region.



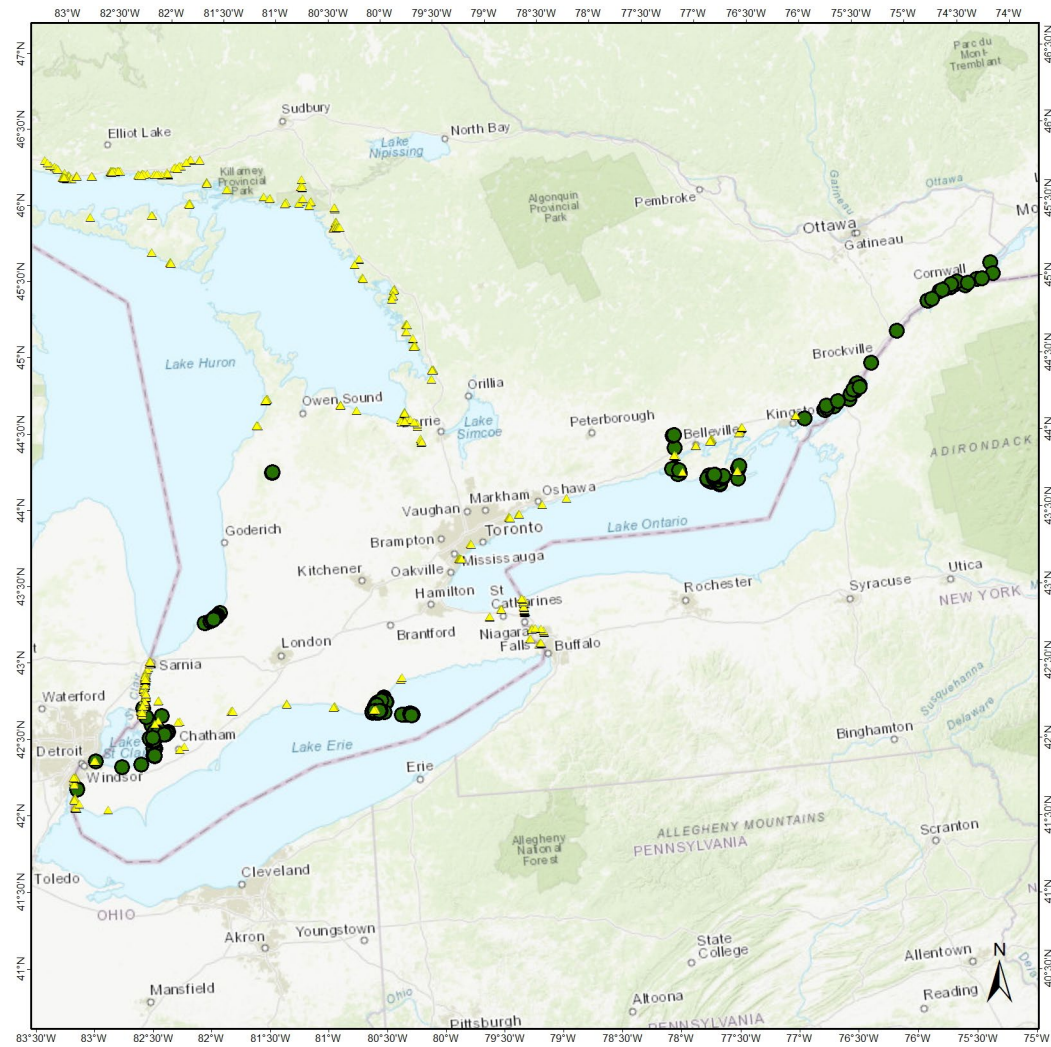


Figure A5.34. Spatial distribution of Pugnose Shiner records (1998–2017; green circles) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.

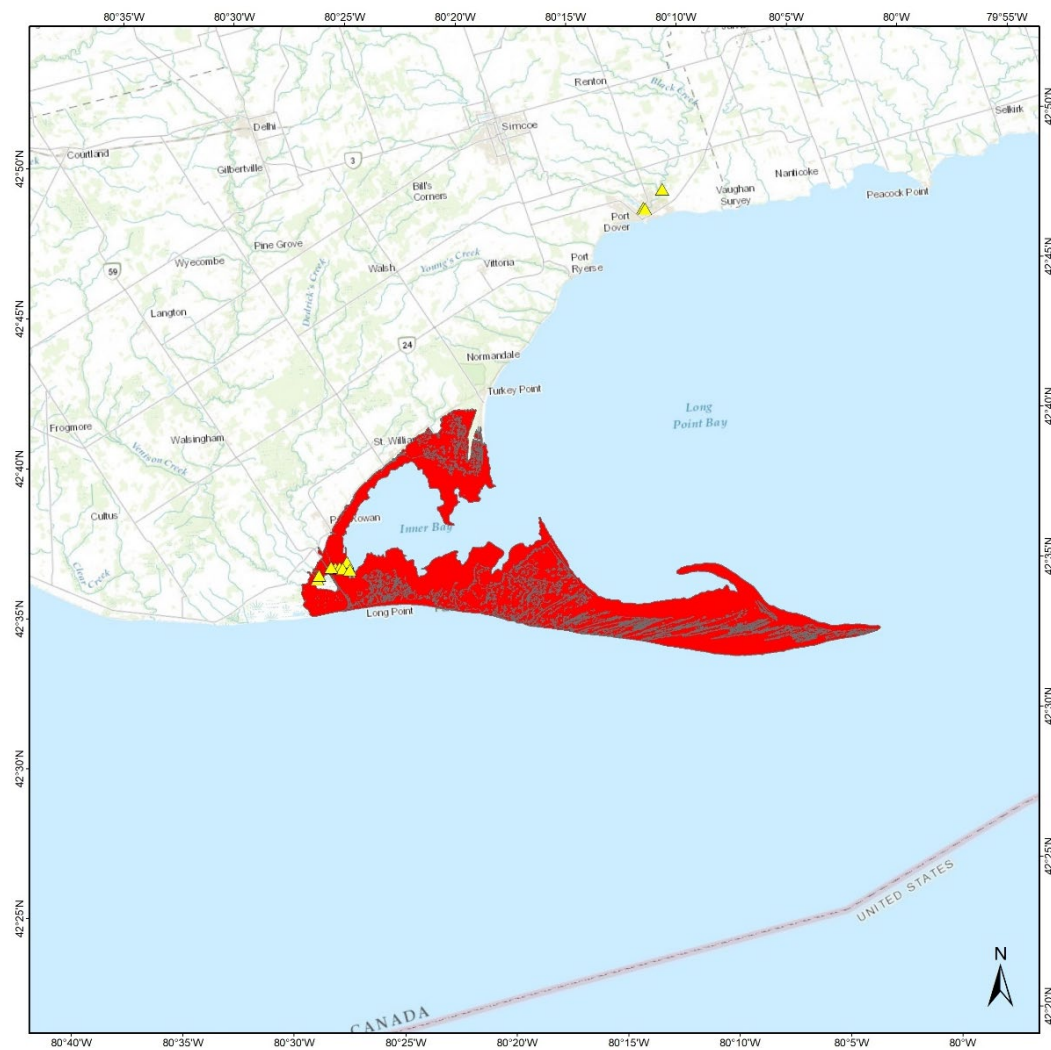


Figure A5.35. Spatial distribution of Pugnose Shiner critical habitat (red shaded area) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.

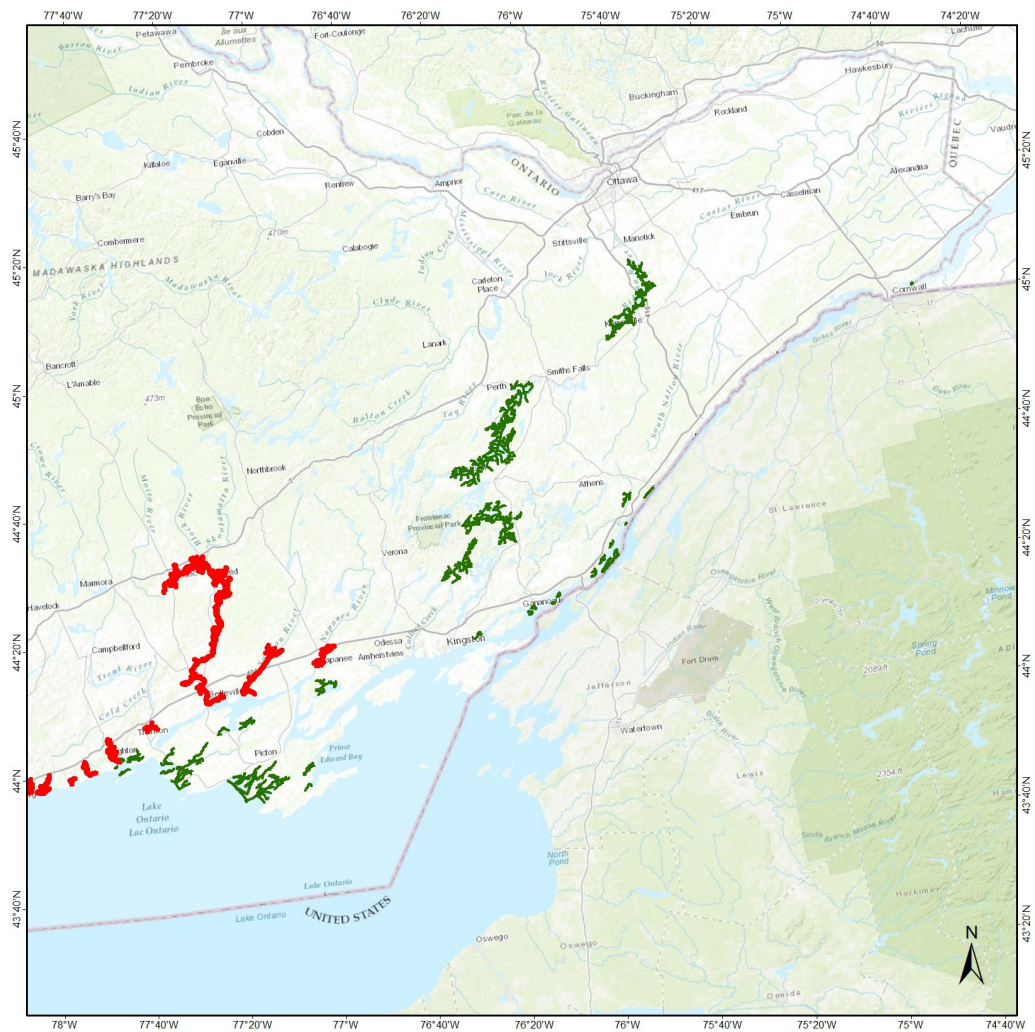


Figure A5.36. Spatial distribution of Bridle Shiner (1998–2017; green shaded area) and larval Sea Lamprey (2011–2017; red shaded area) in Ontario's Great Lakes region.



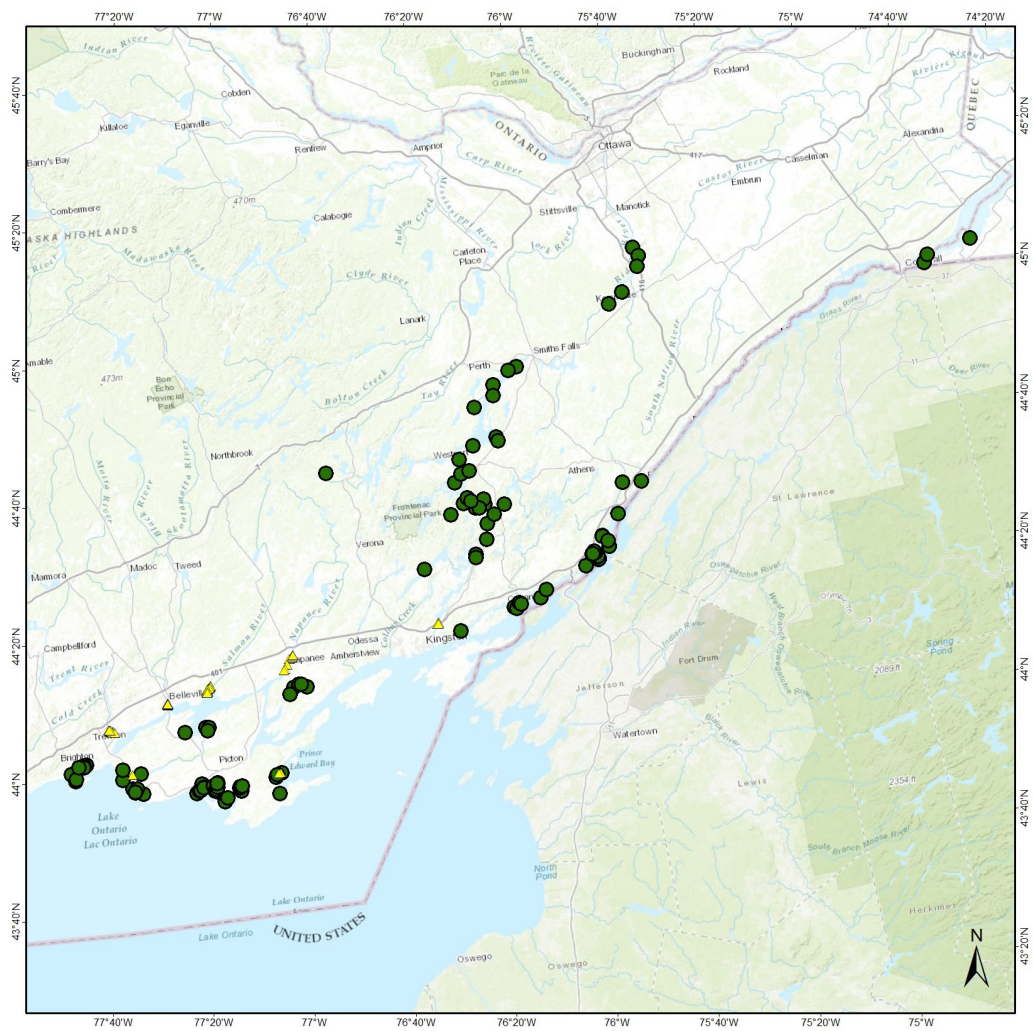


Figure A5.37. Spatial distribution of Bridle Shiner records (1998–2017; green circles) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.

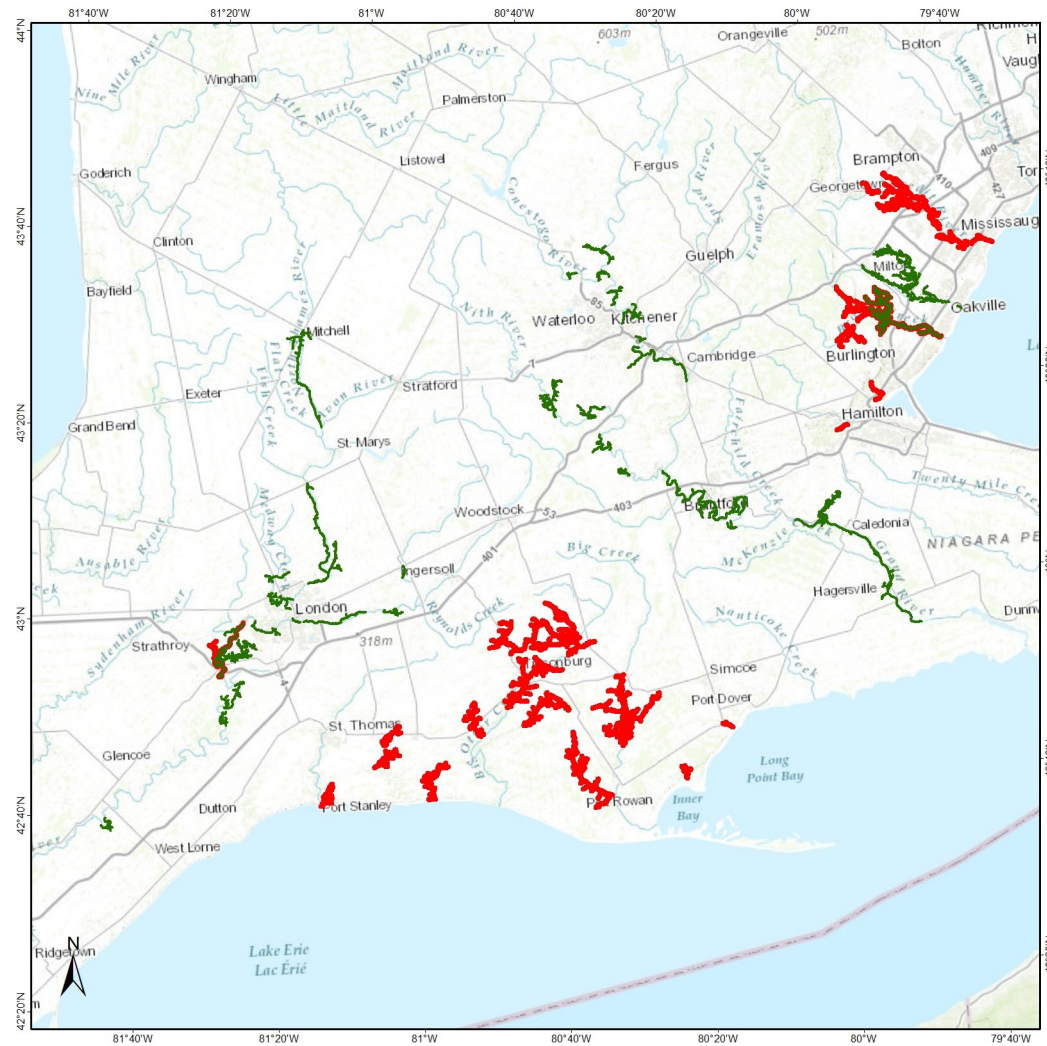


Figure A5.38. Spatial distribution of Silver Shiner (1998–2017; green shaded area) and larval Sea Lamprey (2011–2017; red shaded area) in Ontario's Great Lakes region.



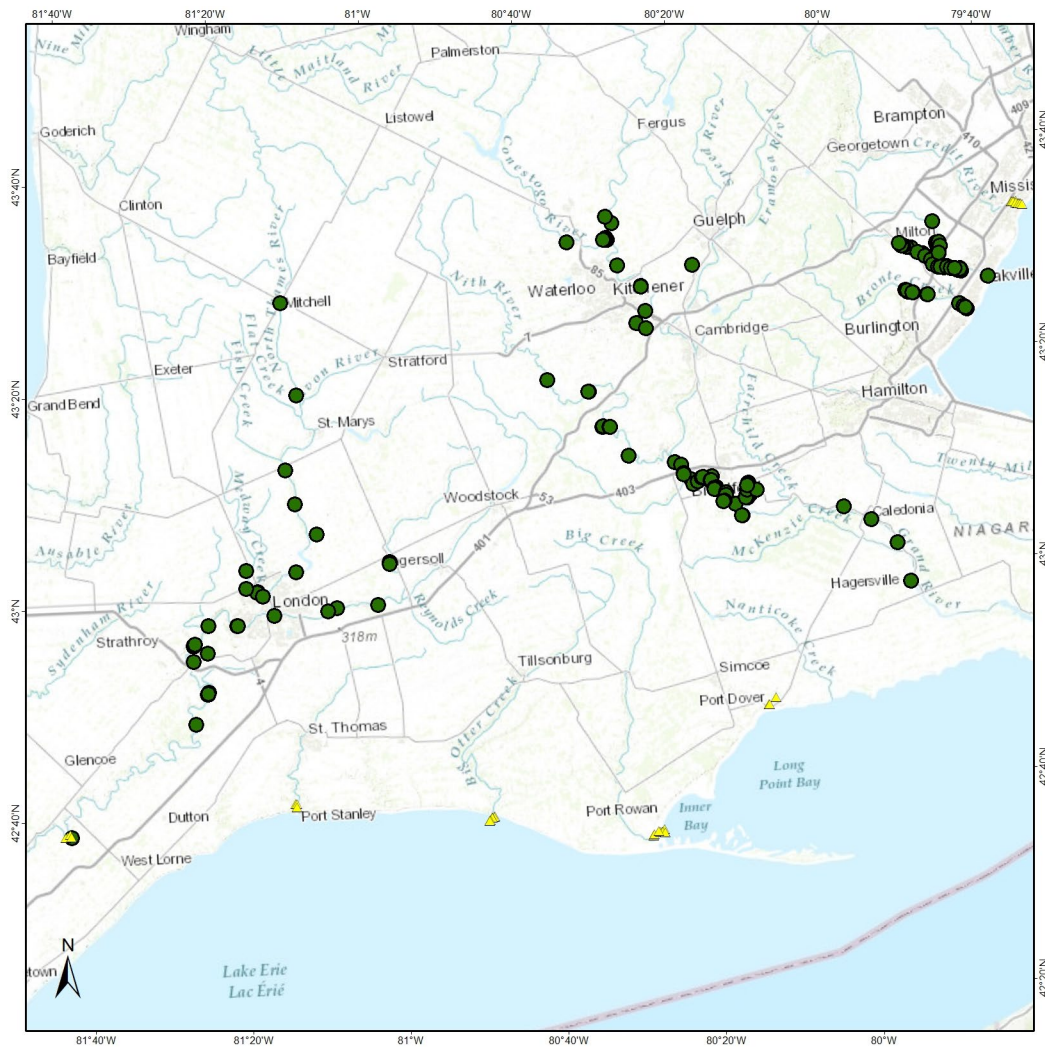


Figure A5.39. Spatial distribution of Silver Shiner records (1998–2017; green circles) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.

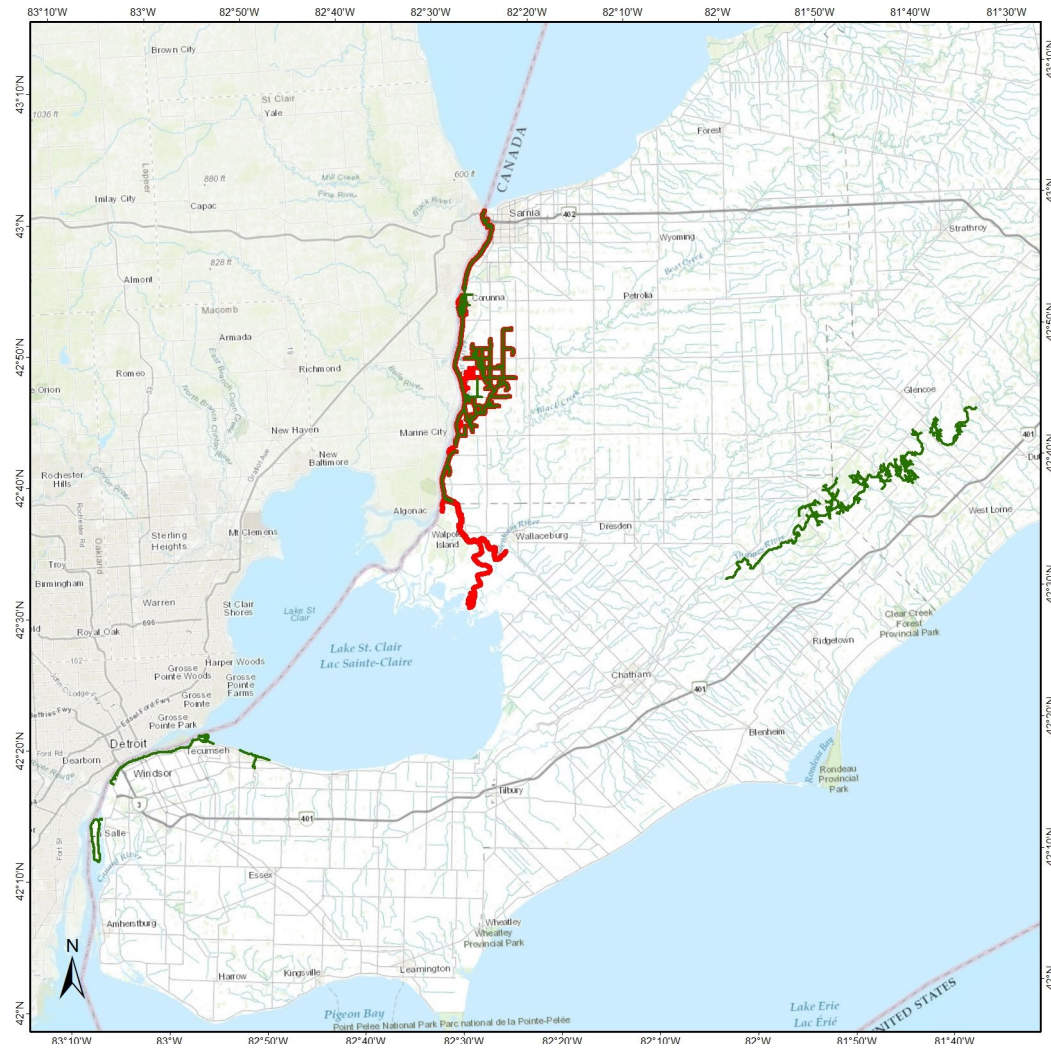


Figure A5.40. Spatial distribution of Northern Madtom (1998–2017; green shaded area) and larval Sea Lamprey (2011–2017; red shaded area) in Ontario's Great Lakes region.

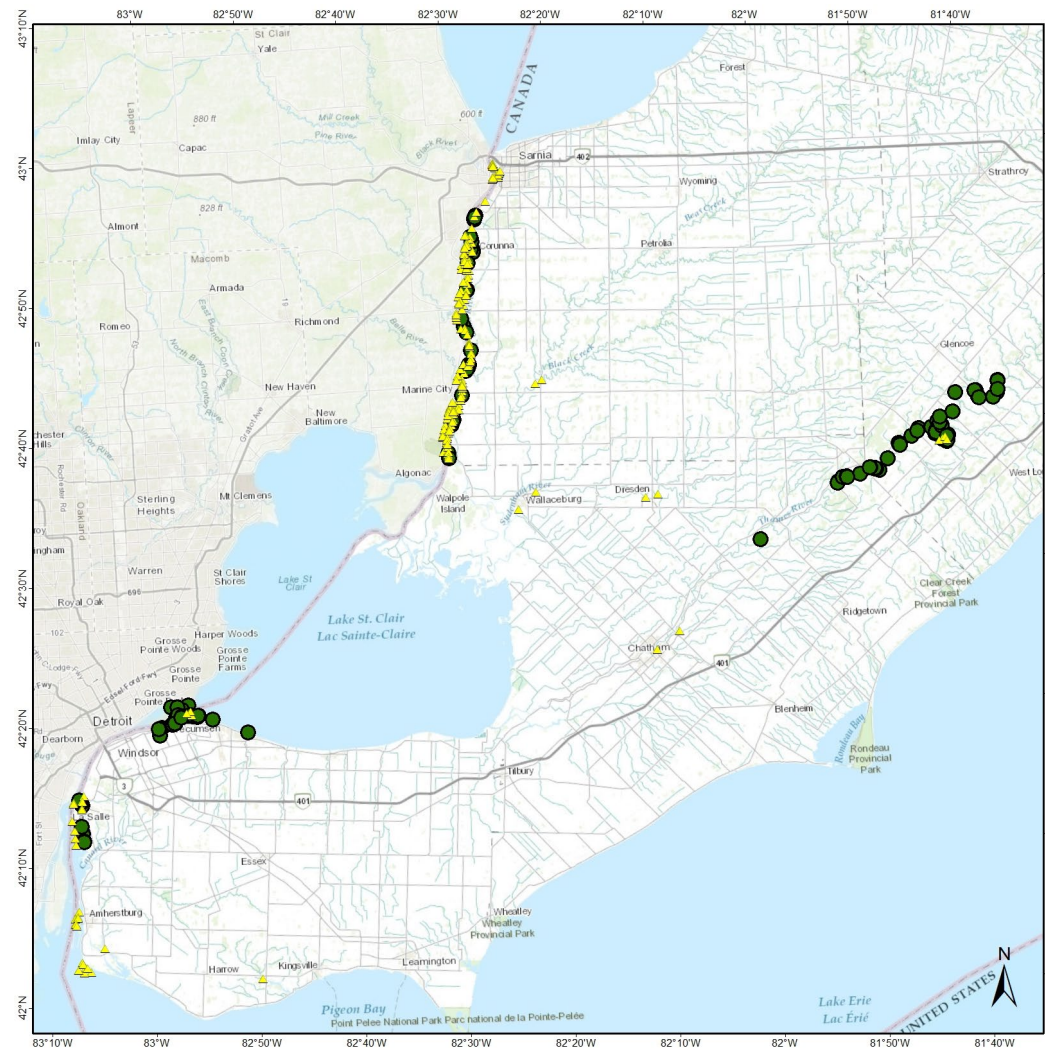


Figure A5.41. Spatial distribution of Northern Madtom records (1998–2017; green circles) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.



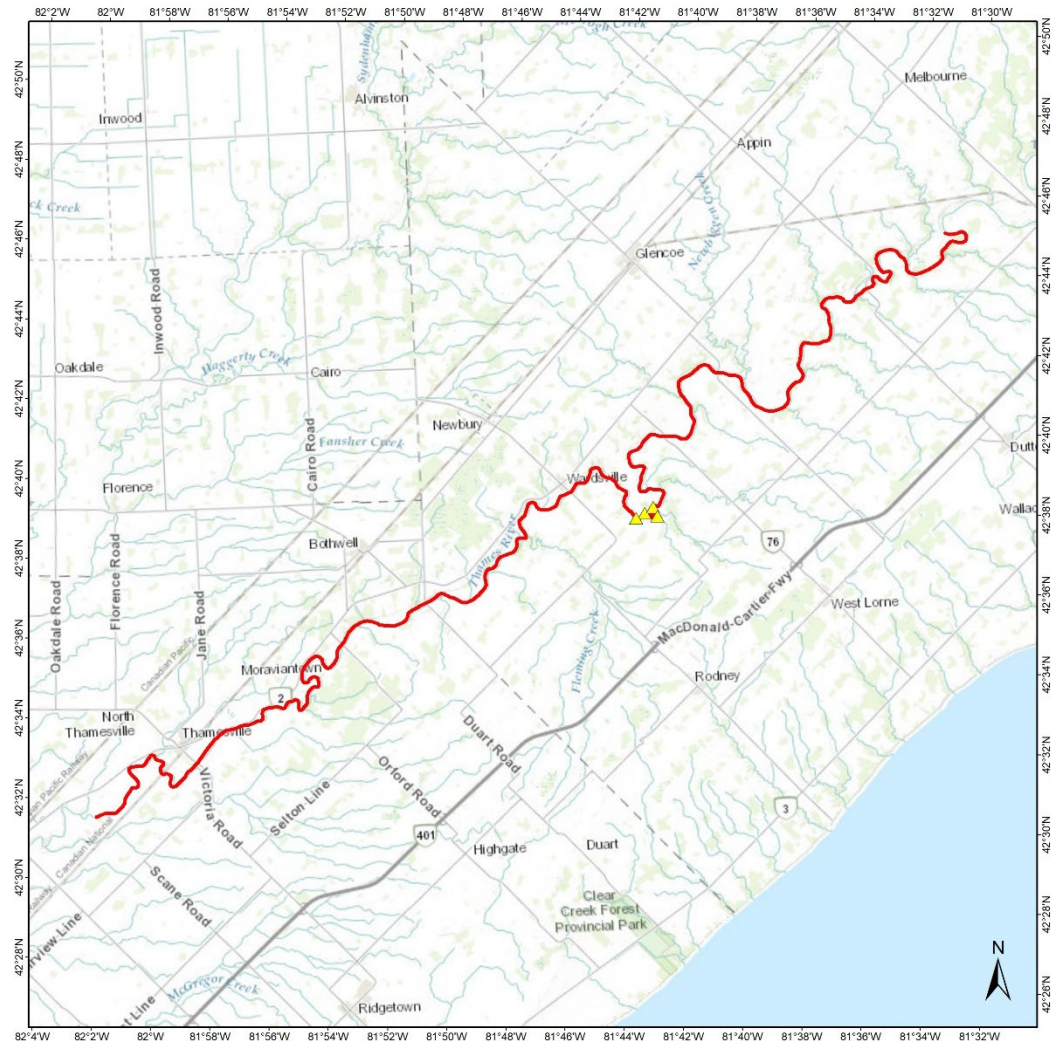


Figure A5.42. Spatial distribution of Northern Madtom critical habitat (red line) in the Thames River, ON and Bayluscide application locations (2011–2017; yellow triangles).

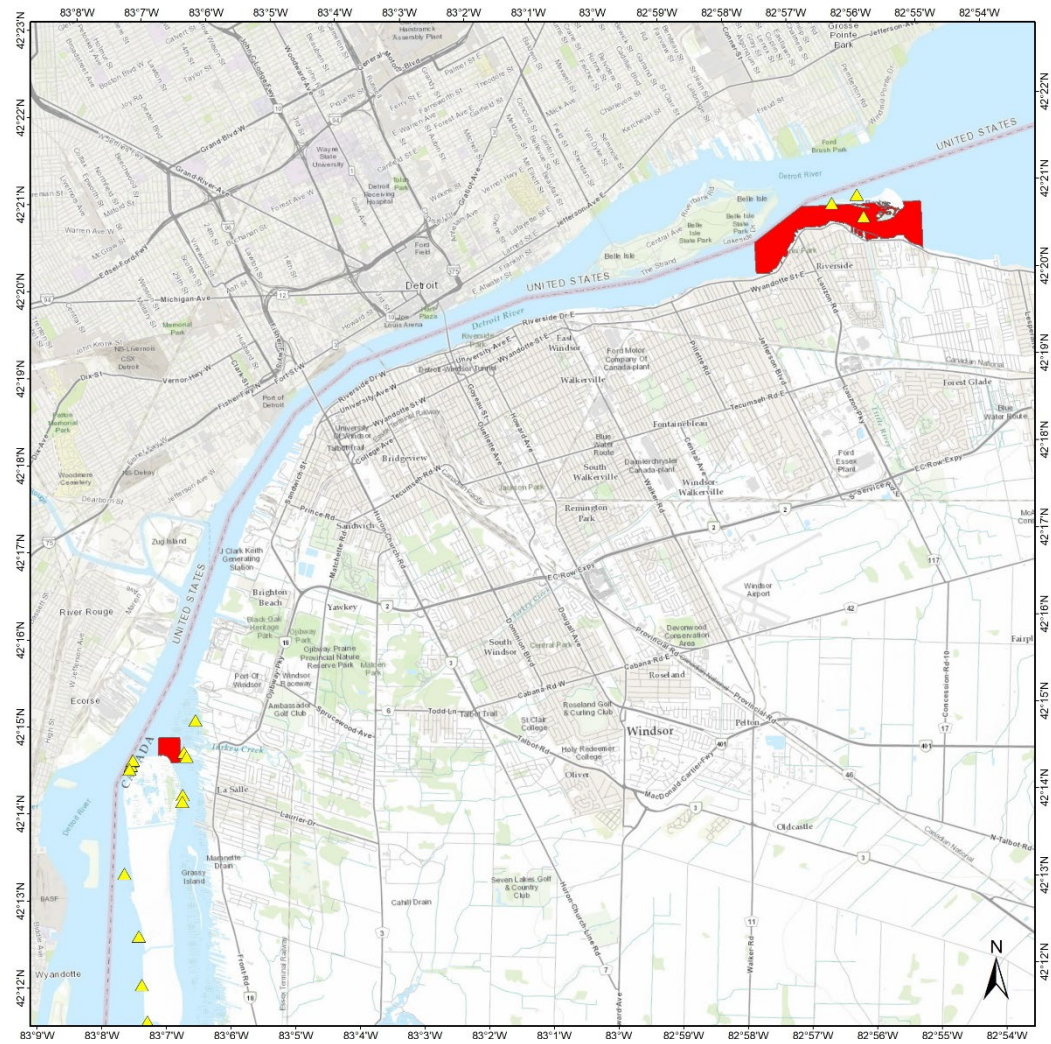


Figure A5.43. Spatial distribution of Northern Madtom critical habitat (red shaded area) in the Detroit River, ON and Bayluscide application locations (2011–2017; yellow triangles).



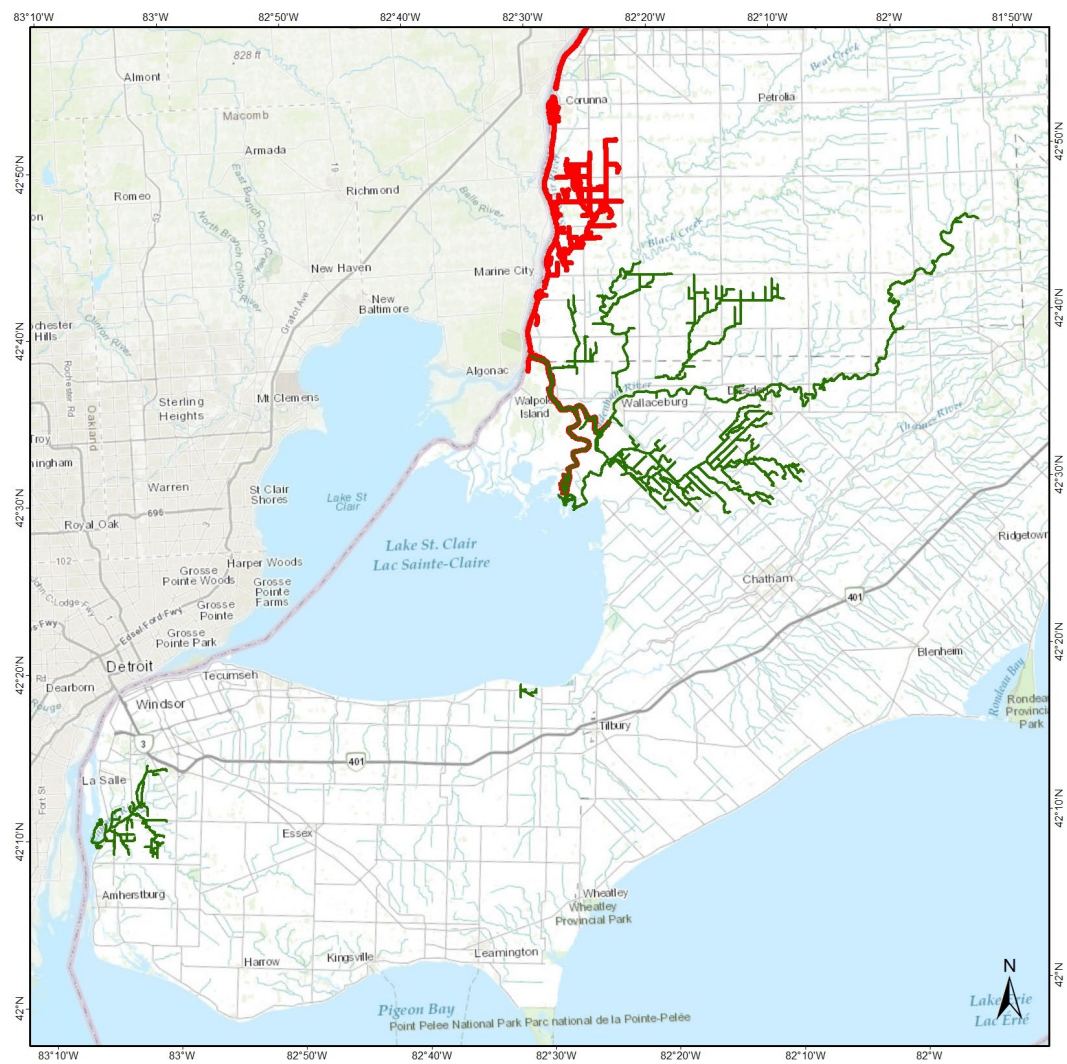


Figure A5.44. Spatial distribution of Pugnose Minnow (1998–2017; green lines) and larval Sea Lamprey (2011–2017; red lines) in Ontario's Great Lakes region.



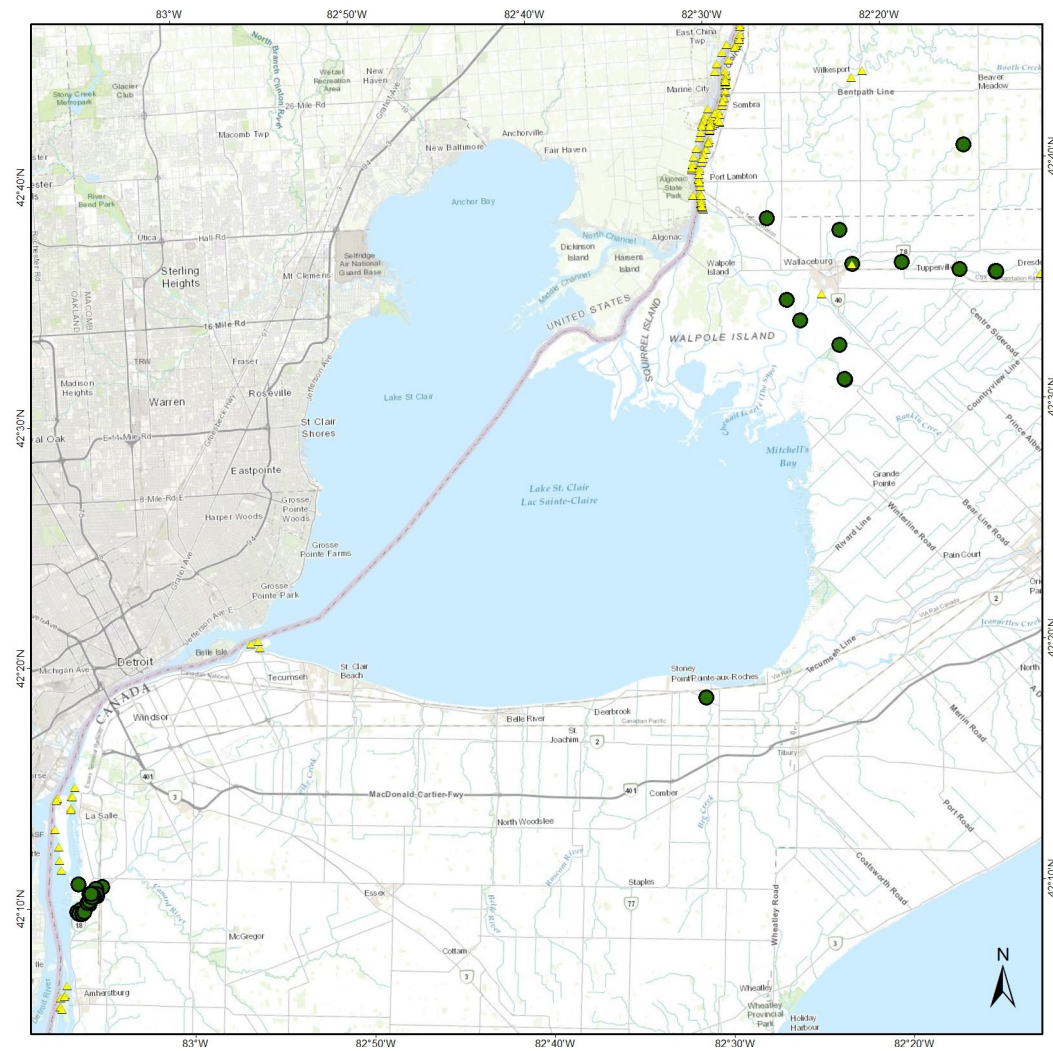


Figure A5.45. Spatial distribution of Pugnose Minnow (1998–2017; green circles) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.

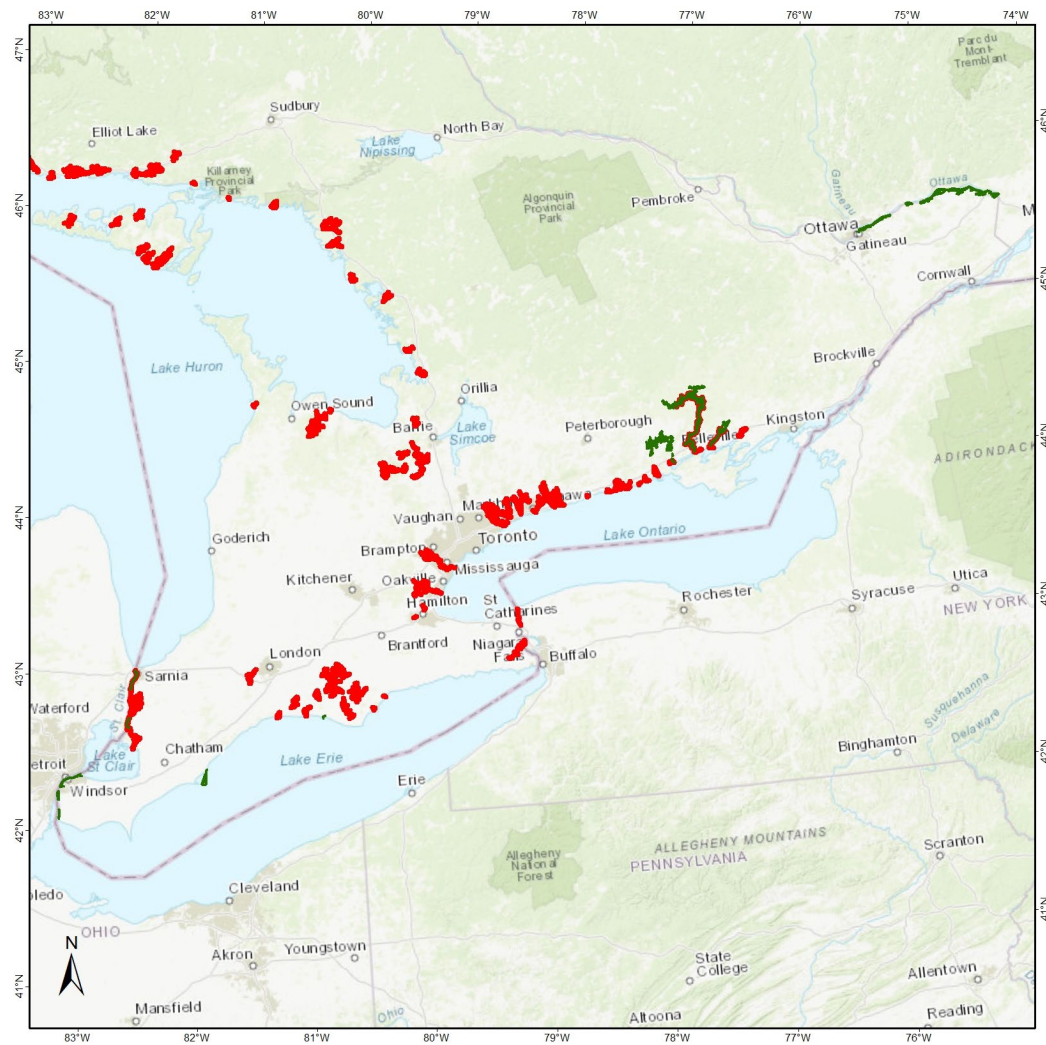


Figure A5.46. Spatial distribution of Channel Darter (1998–2017; green shaded area) and larval Sea Lamprey (2011–2017; red shaded area) in Ontario's Great Lakes region.

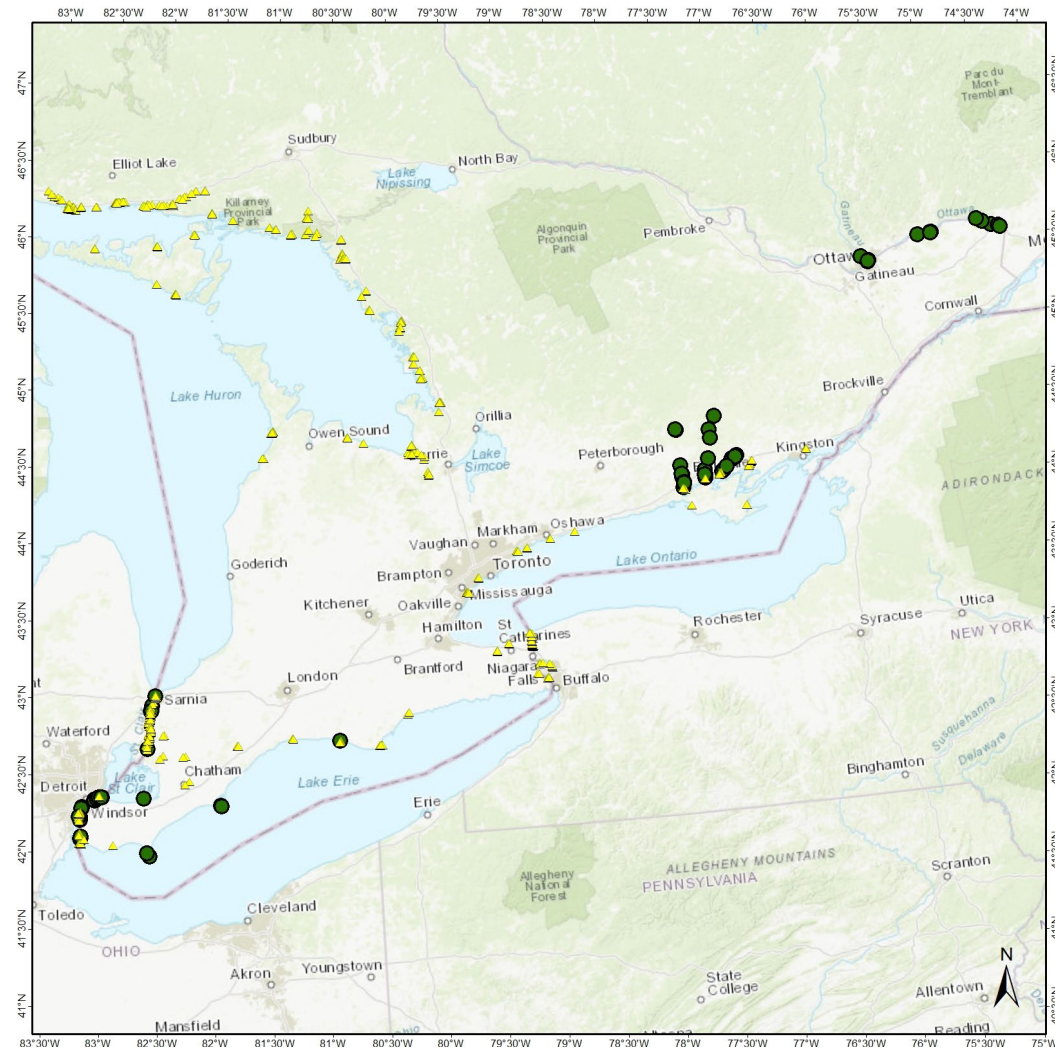


Figure A5.47. Spatial distribution of Channel Darter records (1998–2017; green circles) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.





Figure A5.48. Spatial distribution of Channel Darter critical habitat (red lines and shaded area) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.

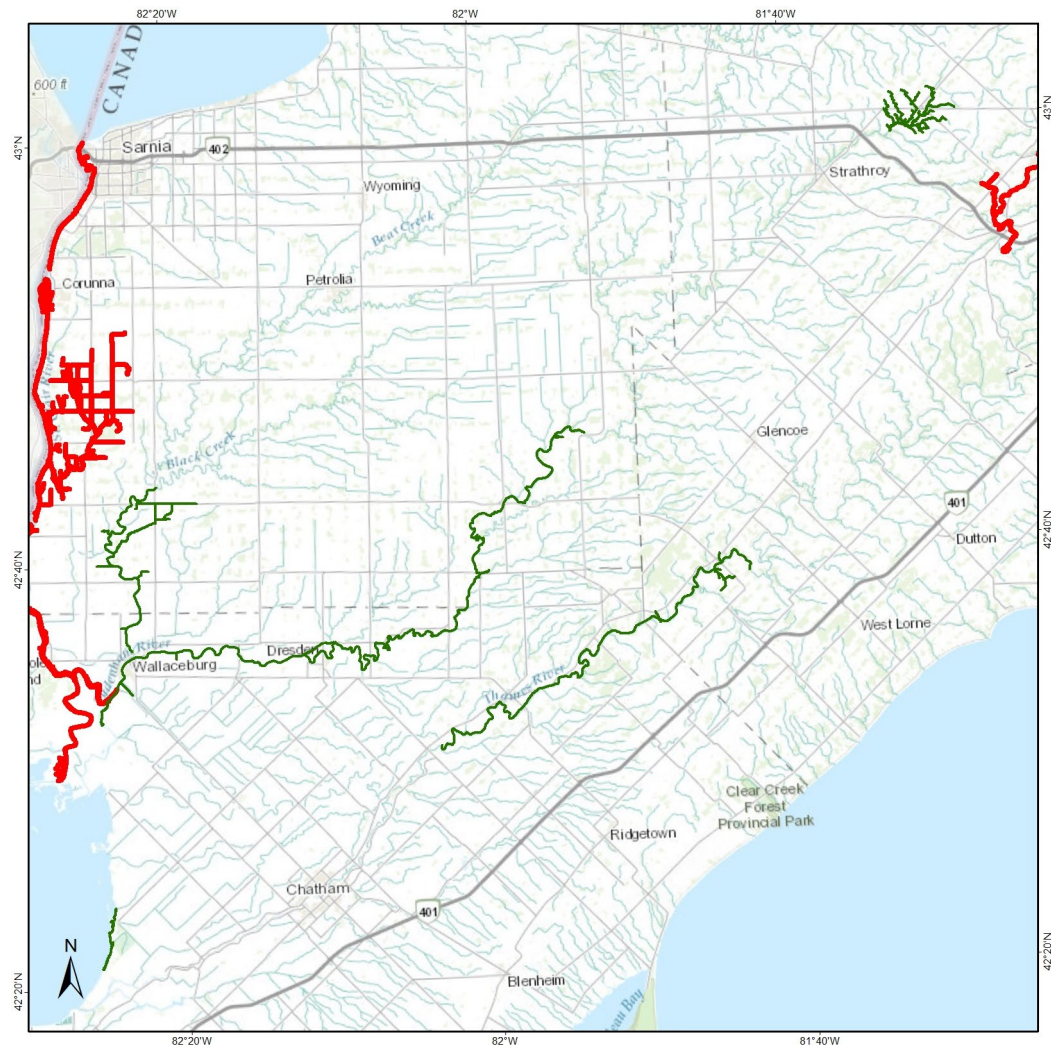


Figure A5.49. Spatial distribution of River Darter (1998–2017; green lines) and larval Sea Lamprey (2011–2017; red lines) in Ontario's Great Lakes region.



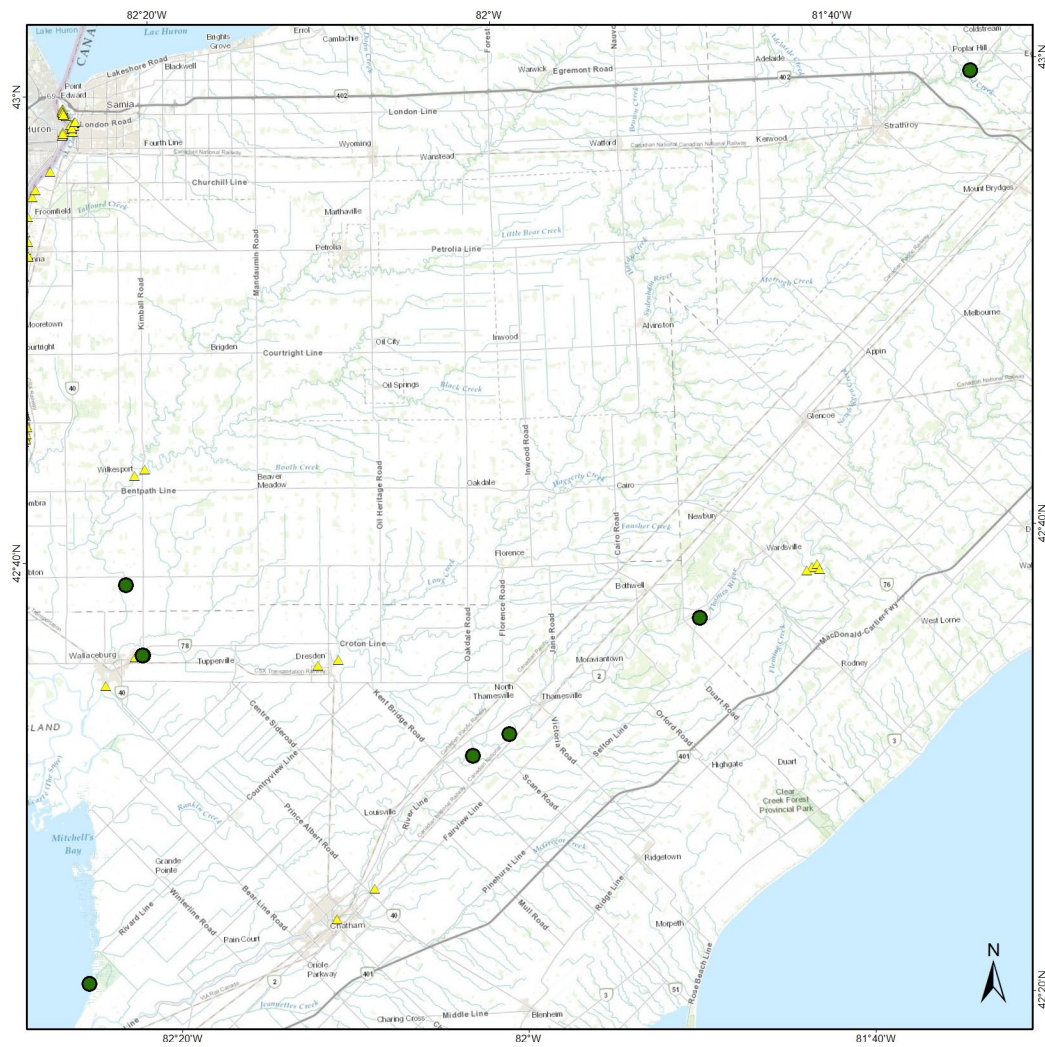


Figure A5.50. Spatial distribution of River Darter records (1998–2017; green circles) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.



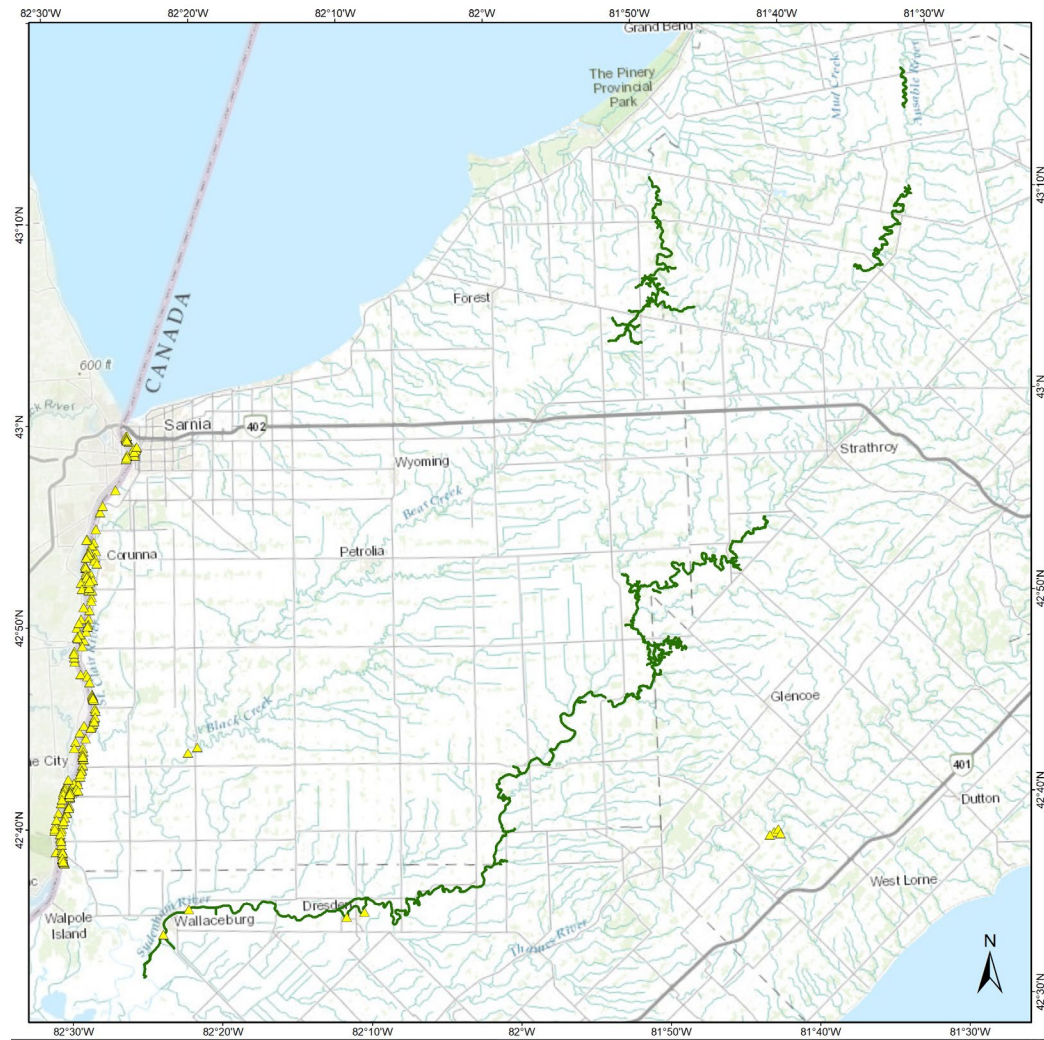


Figure A5.51. Spatial distribution of Northern Riffleshell (1998–2017; green lines) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.

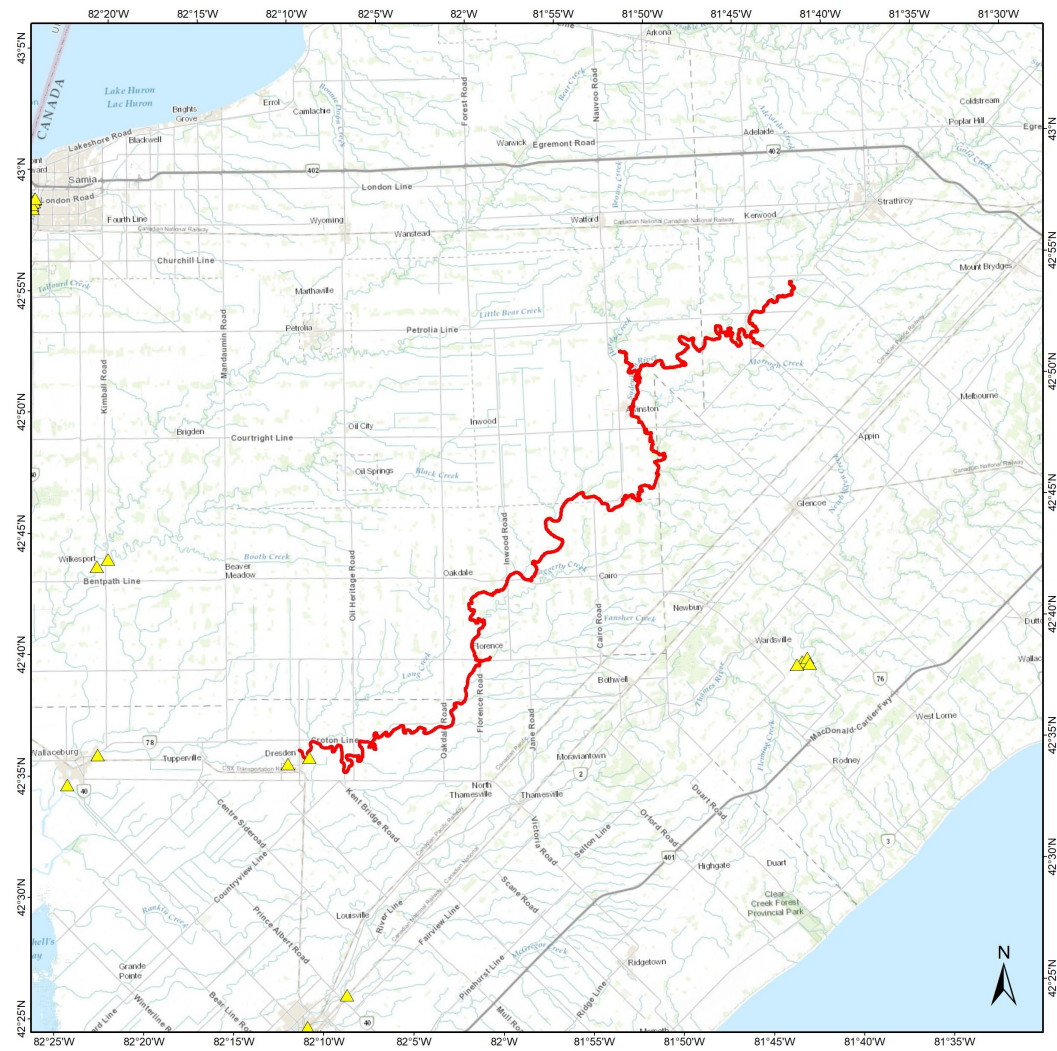


Figure A5.52. Spatial distribution of Northern Riffleshell critical habitat (proposed, red lines) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.



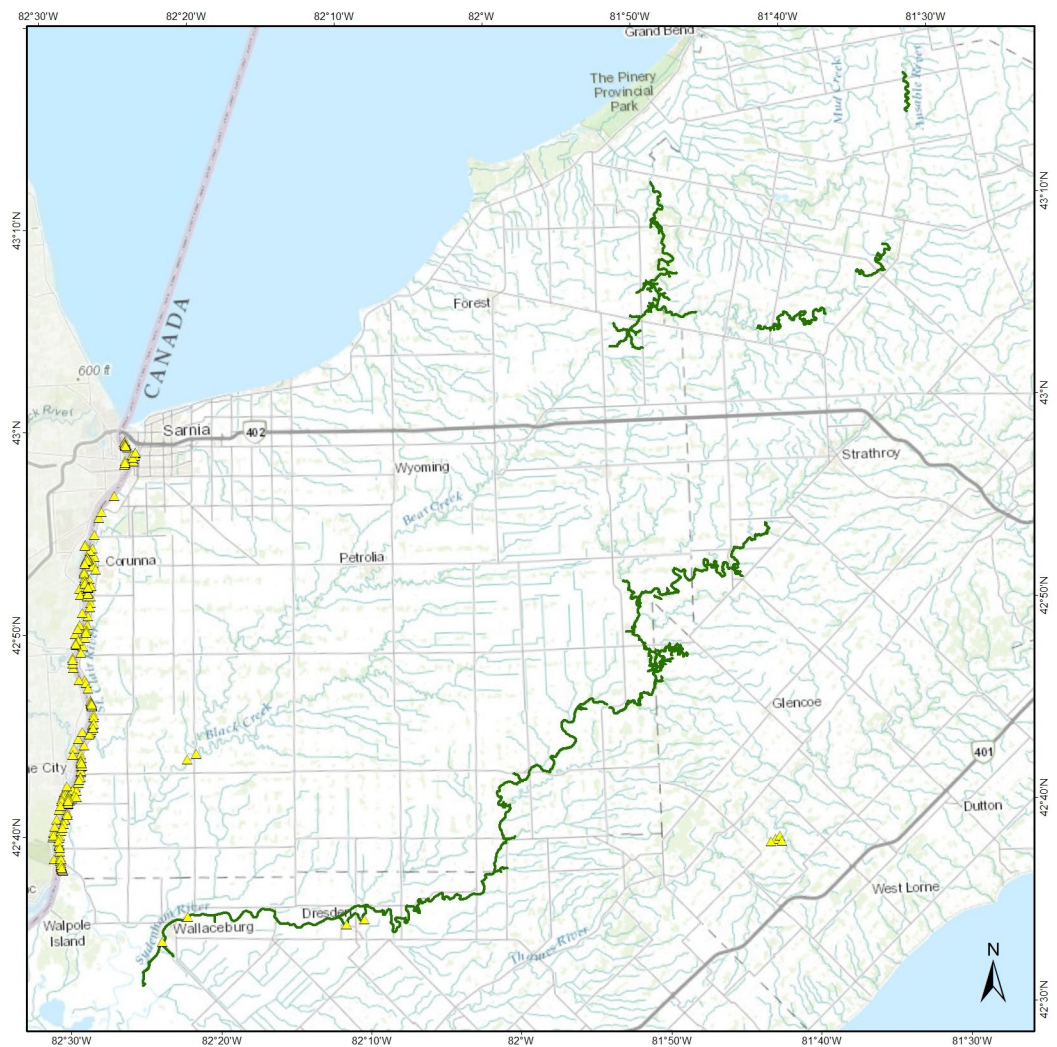


Figure A5.53. Spatial distribution of Snuffbox (1998–2017; green lines) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.

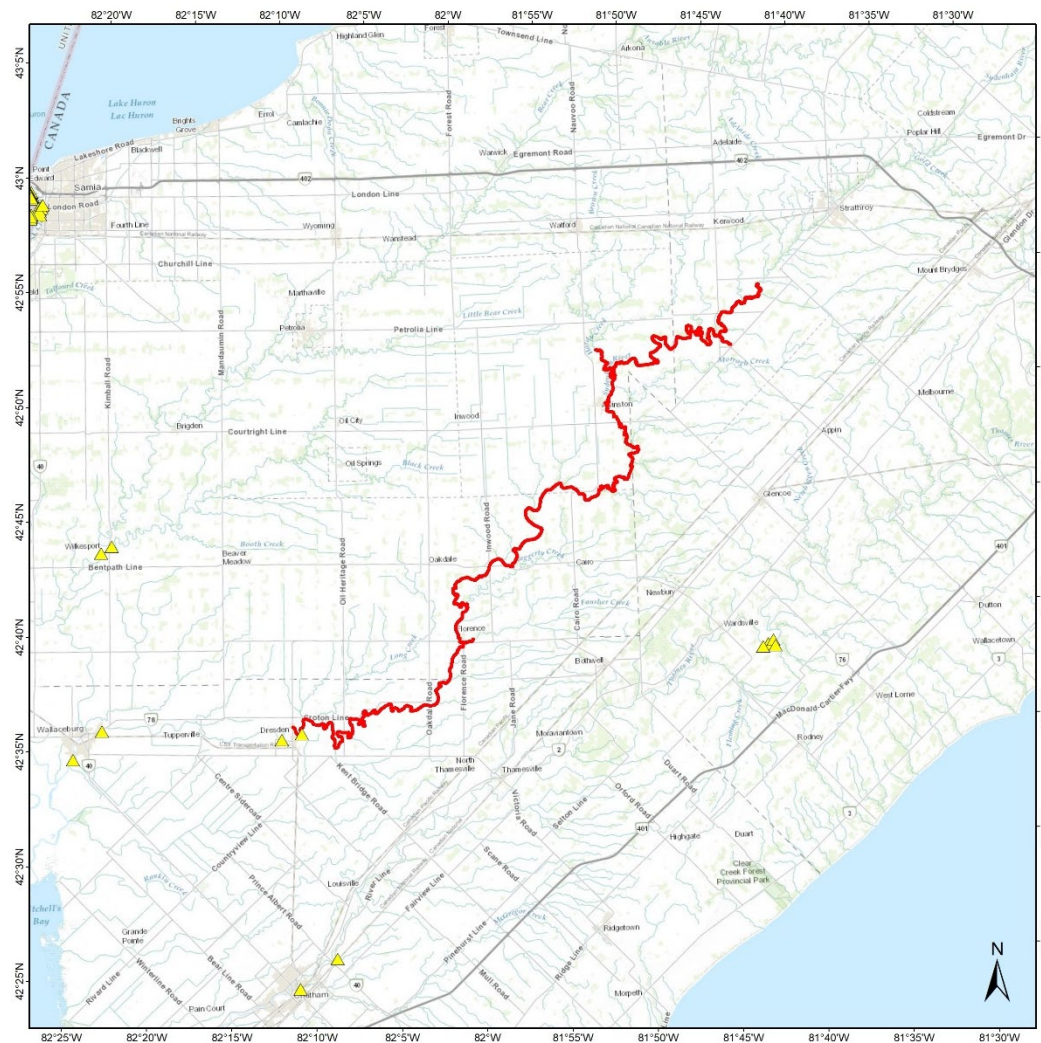


Figure A5.54. Spatial distribution of Snuffbox critical habitat (proposed; red lines) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.

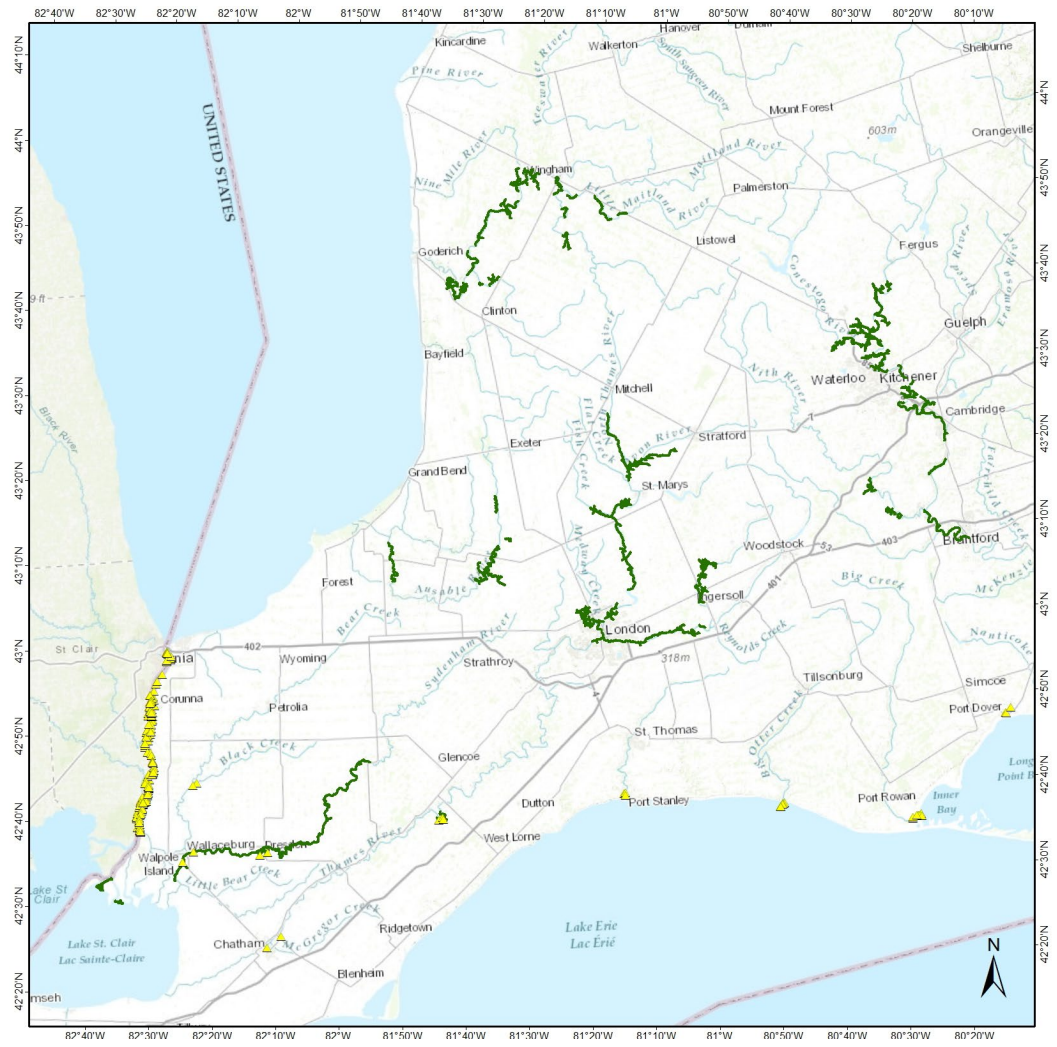


Figure A5.55. Spatial distribution of Wavyrayed lampmussel (1998–2017; green lines and shaded area) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.



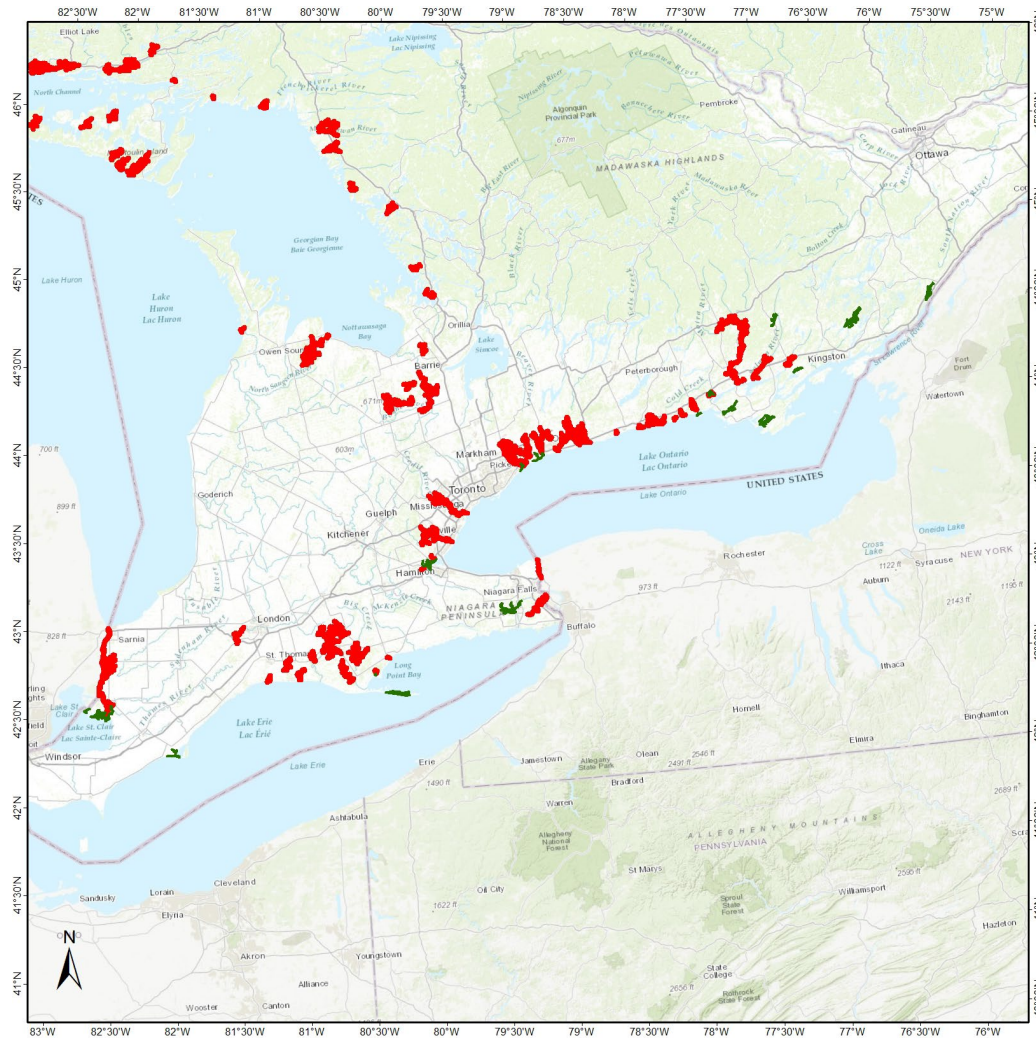


Figure A5.56. Spatial distribution of Eastern Pondmussel (1998–2017; green shaded area) and larval Sea Lamprey (2011–2017; red shaded area) in Ontario's Great Lakes region.



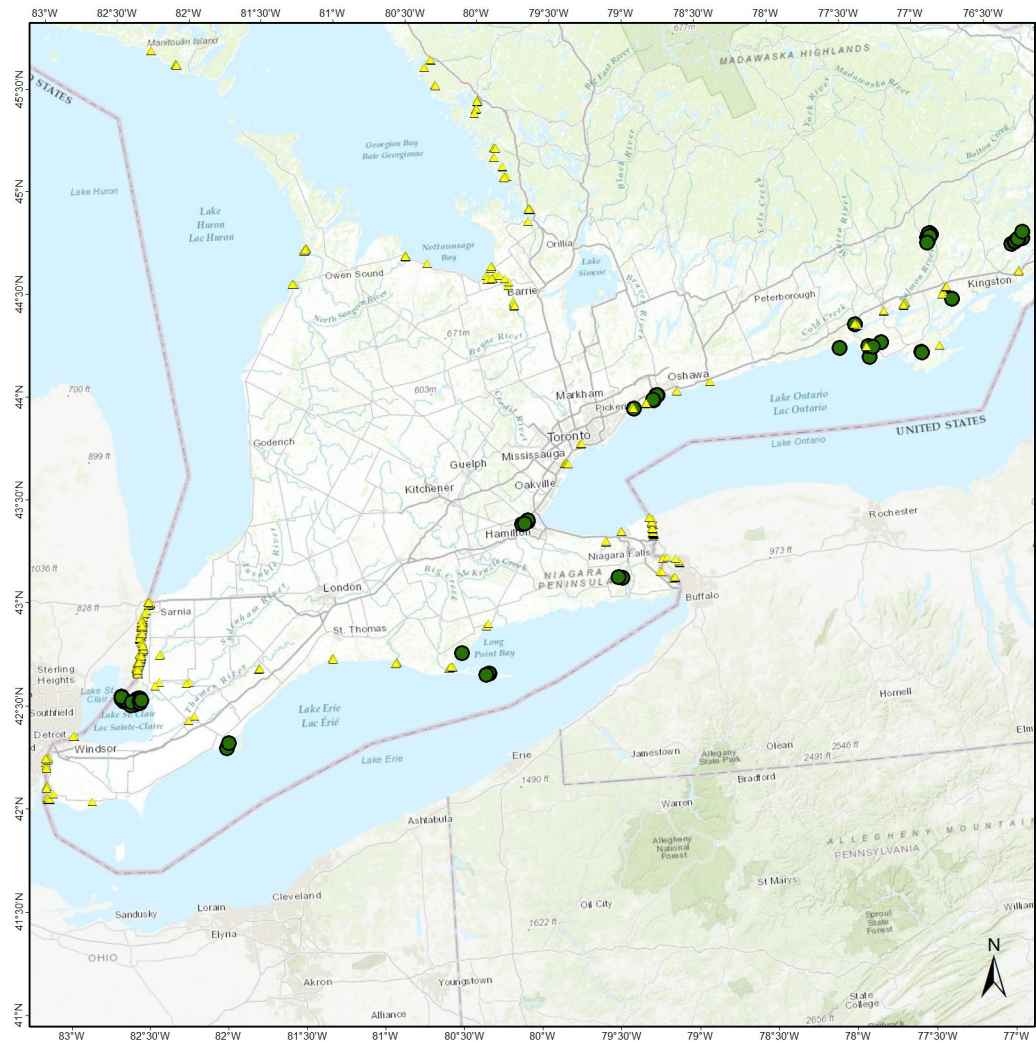


Figure A5.57. Spatial distribution of Eastern Pondmussel records (1998–2017; green circles) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.

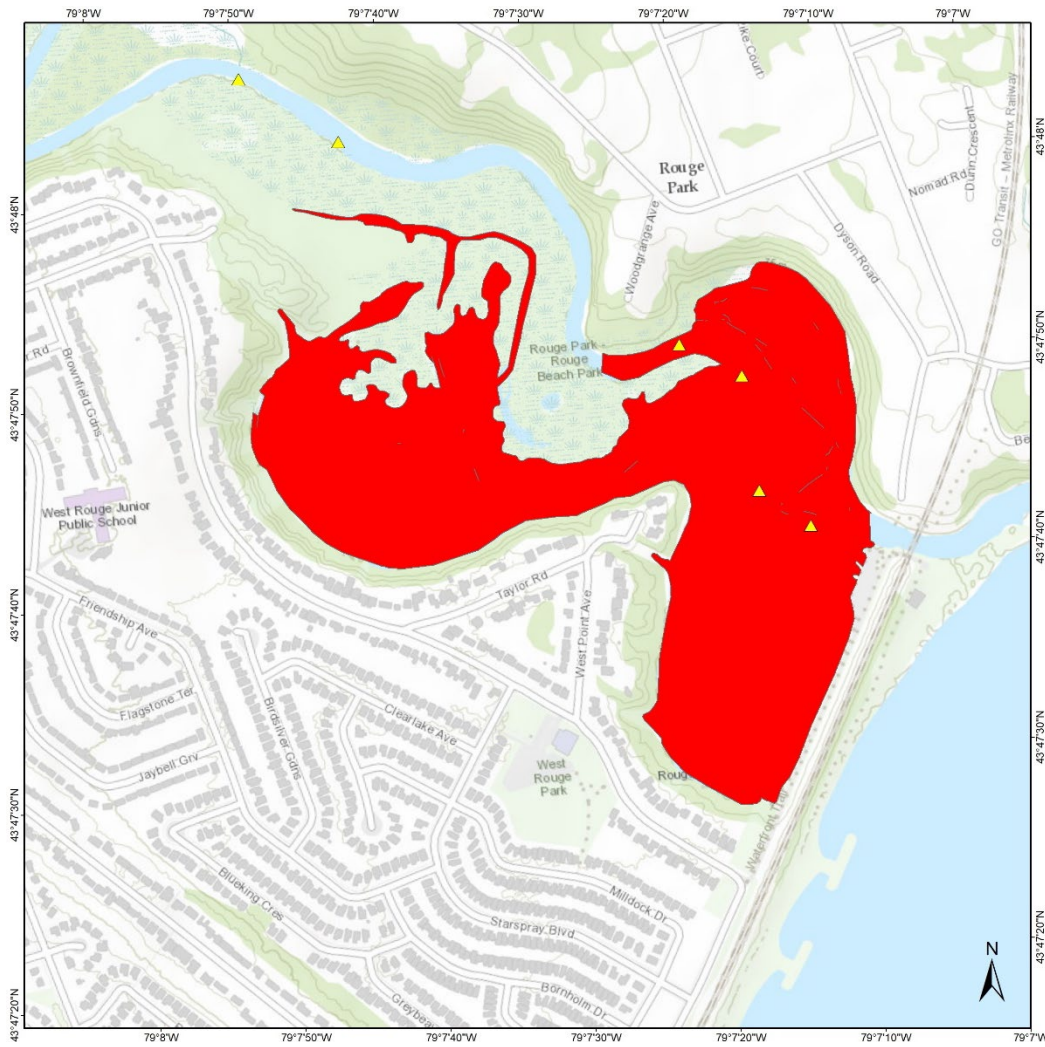


Figure A5.58. Spatial distribution of Eastern Pondmussel critical habitat (proposed; red shaded area) in the Rouge River, ON and Bayluscide application locations (2011–2017; yellow triangles). As Eastern Pondmussel was down-listed to Special Concern on Schedule 1 of SARA in August 2019, critical habitat no longer exists for this species

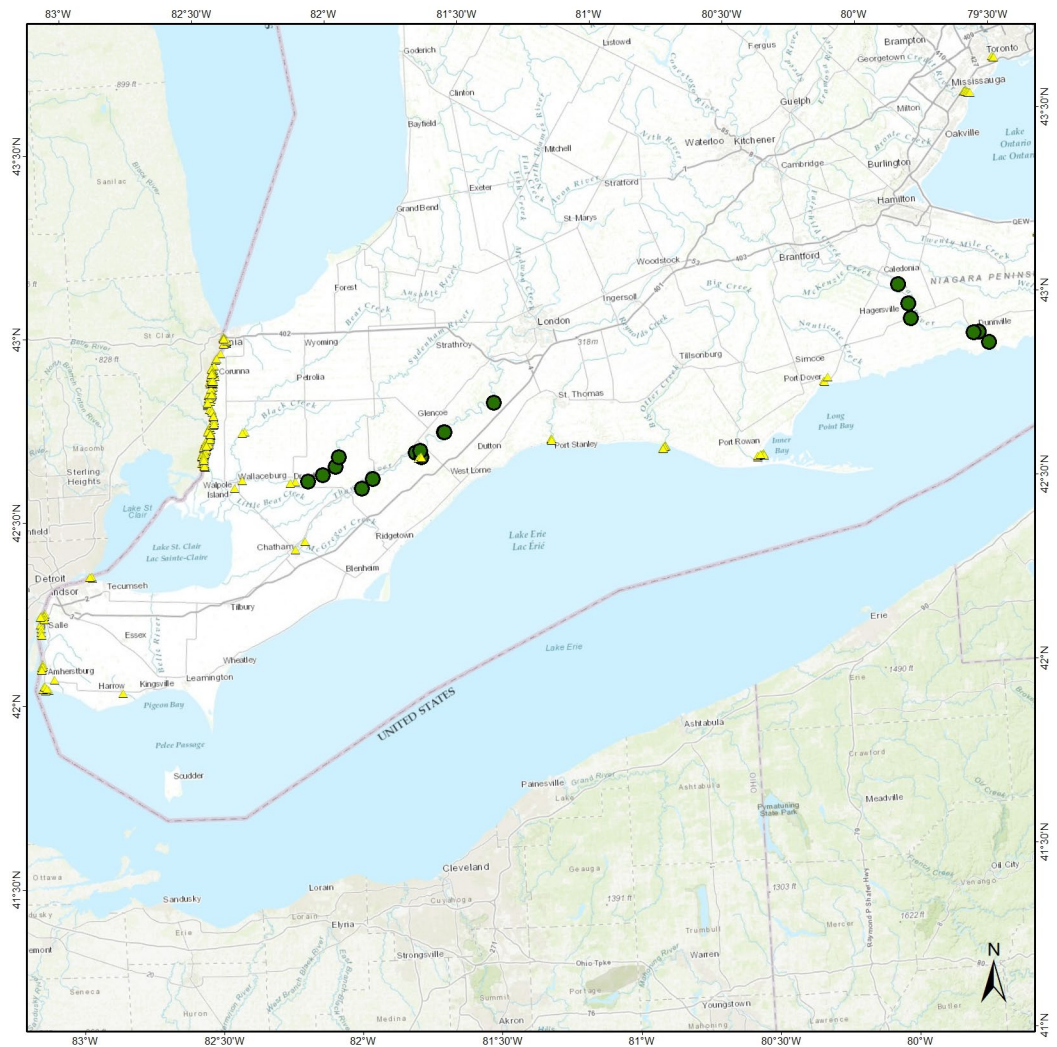


Figure A5.59. Spatial distribution of Threhorn Wartyback records (1998–2017; green circles) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.



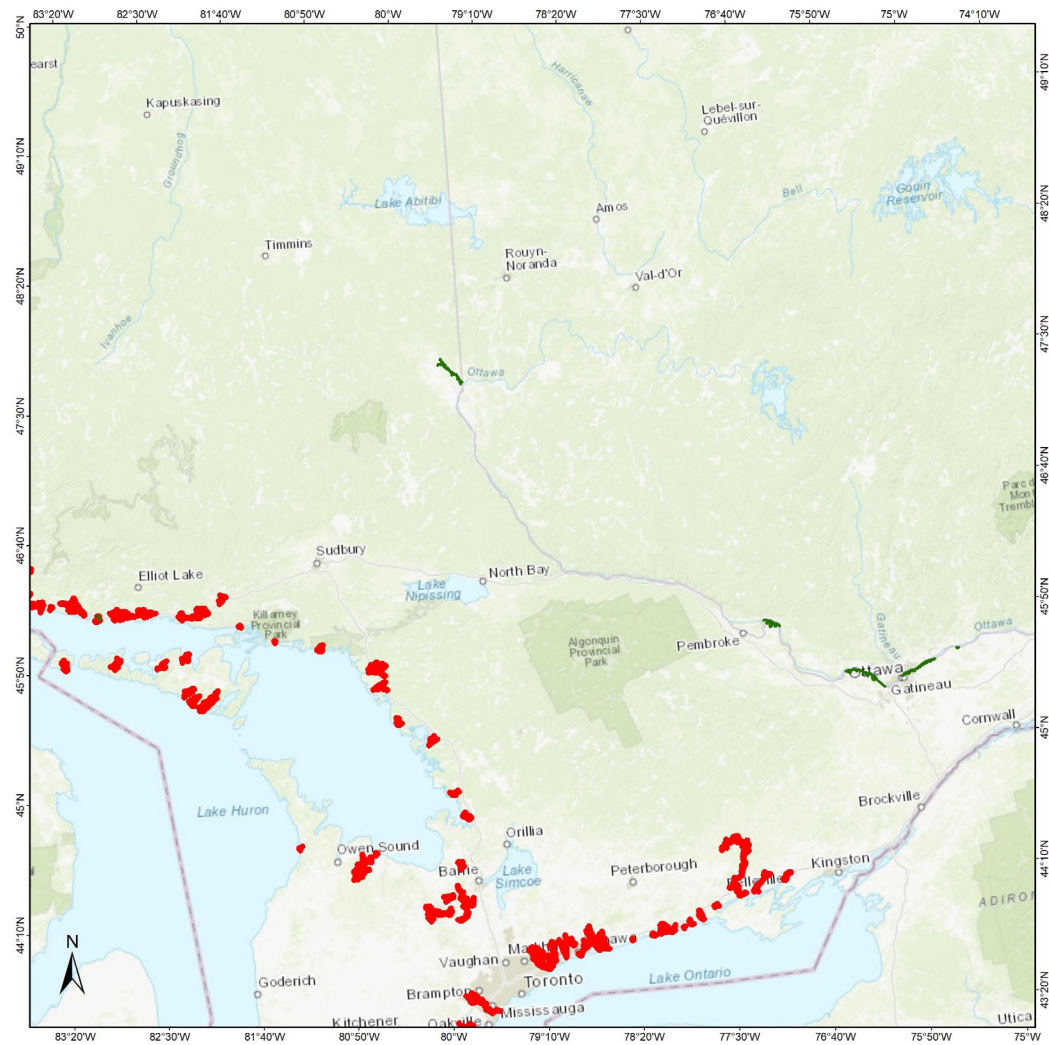


Figure A5.60. Spatial distribution of Hickorynut (1998–2017; green shaded area) and larval Sea Lamprey (2011–2017; red shaded area) in Ontario's Great Lakes region.

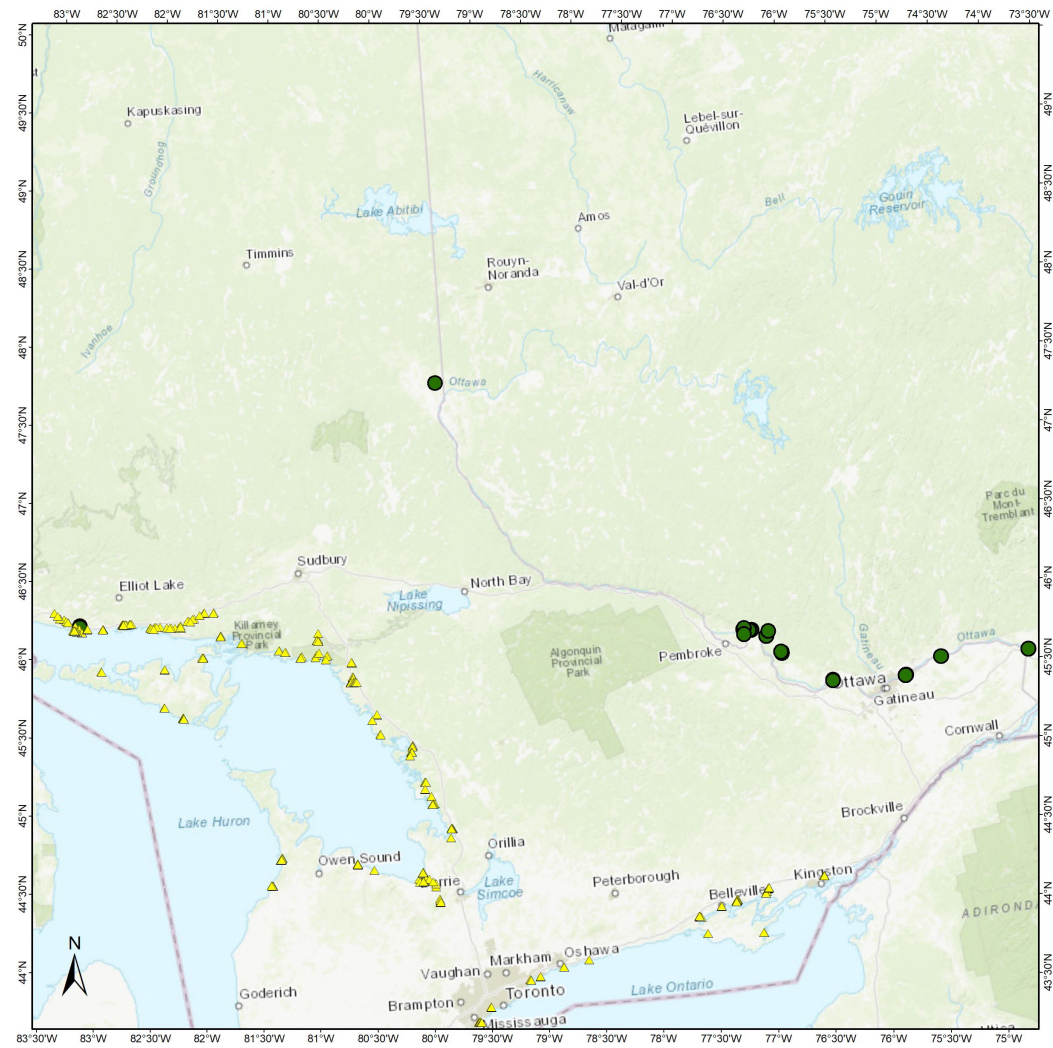


Figure A5.61. Spatial distribution of Hickorynut records (1998–2017; green circles) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.

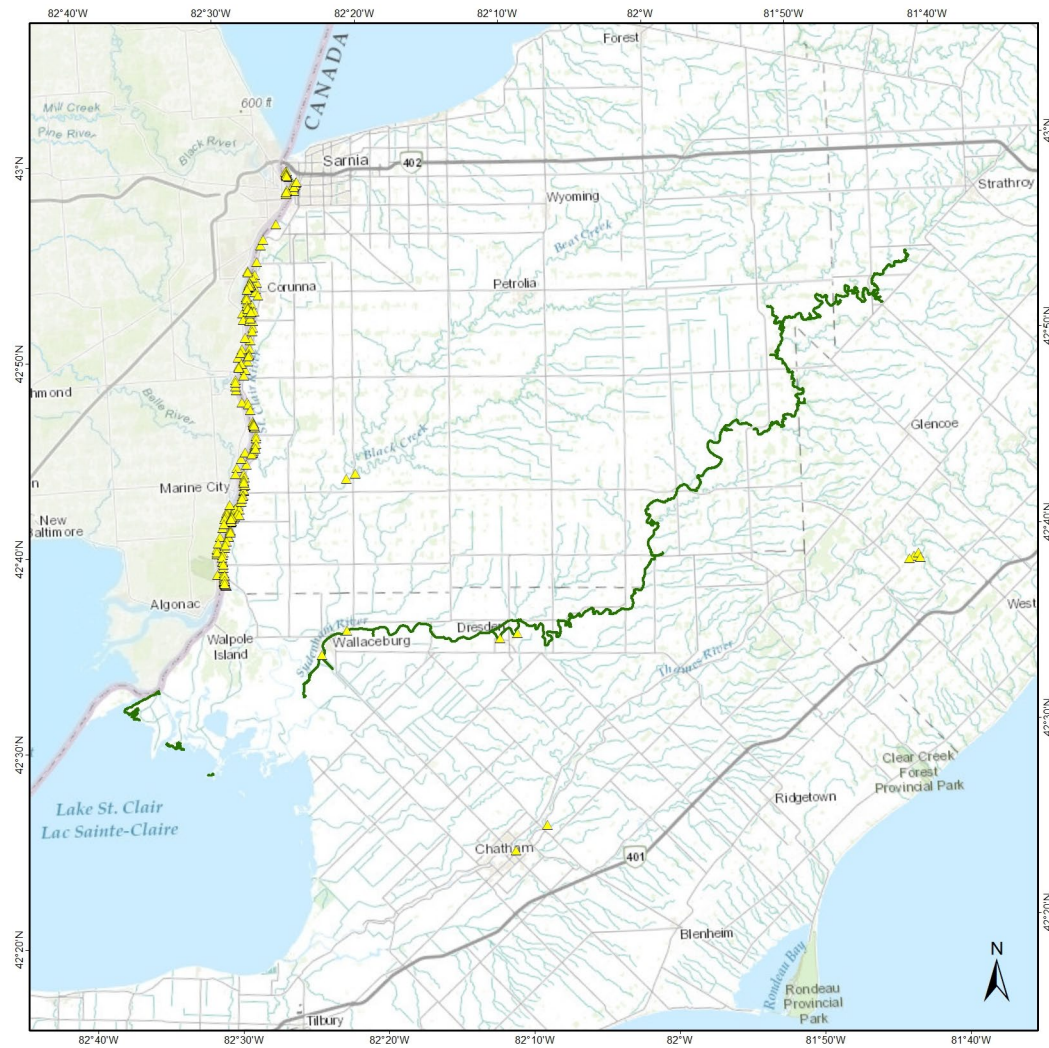


Figure A5.62. Spatial distribution of Round Hickorynut (1998–2017; green lines) and Bayluside application locations (2011–2017; yellow triangles) in Ontario.



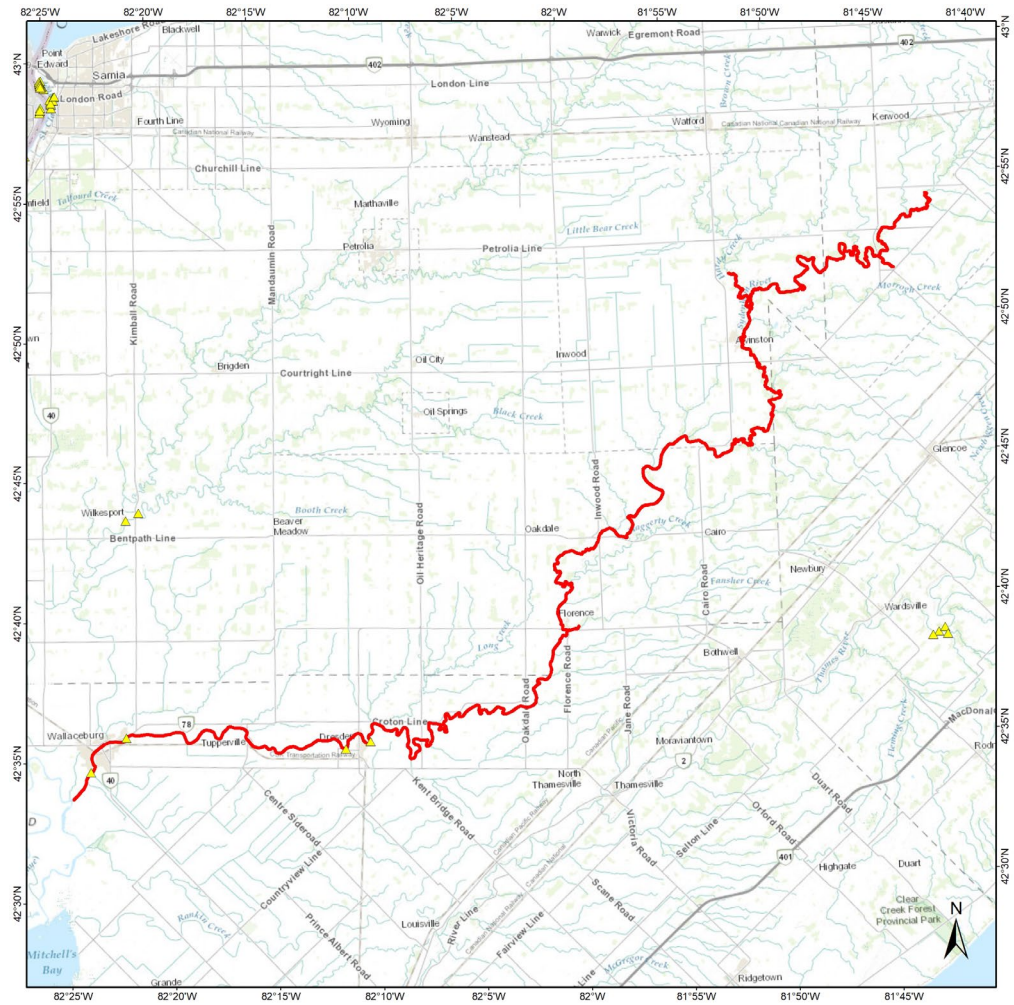


Figure A5.63. Spatial distribution of Round Hickorynut critical habitat (red lines) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.

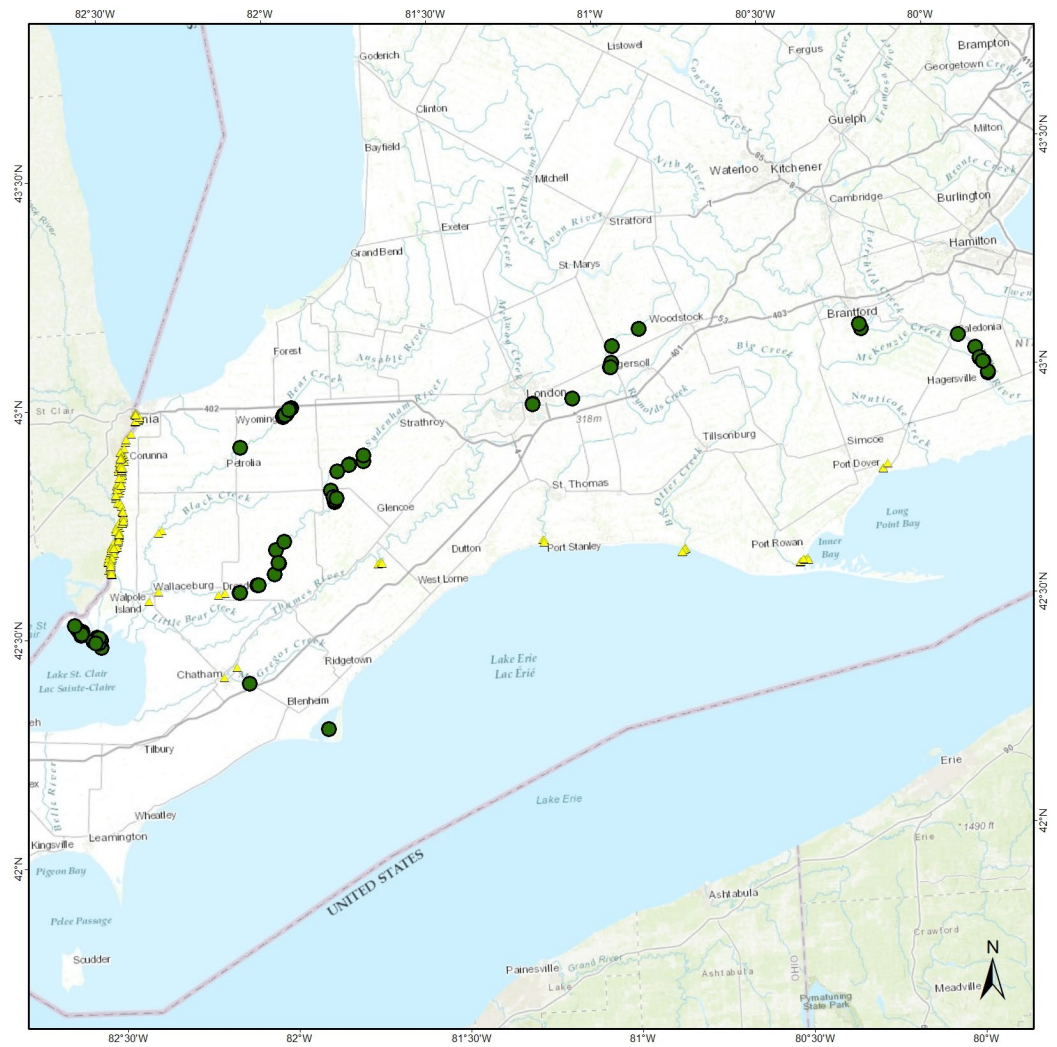


Figure A5.64. Spatial distribution of Round Pigtoe records (1998–2017; green circles) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.

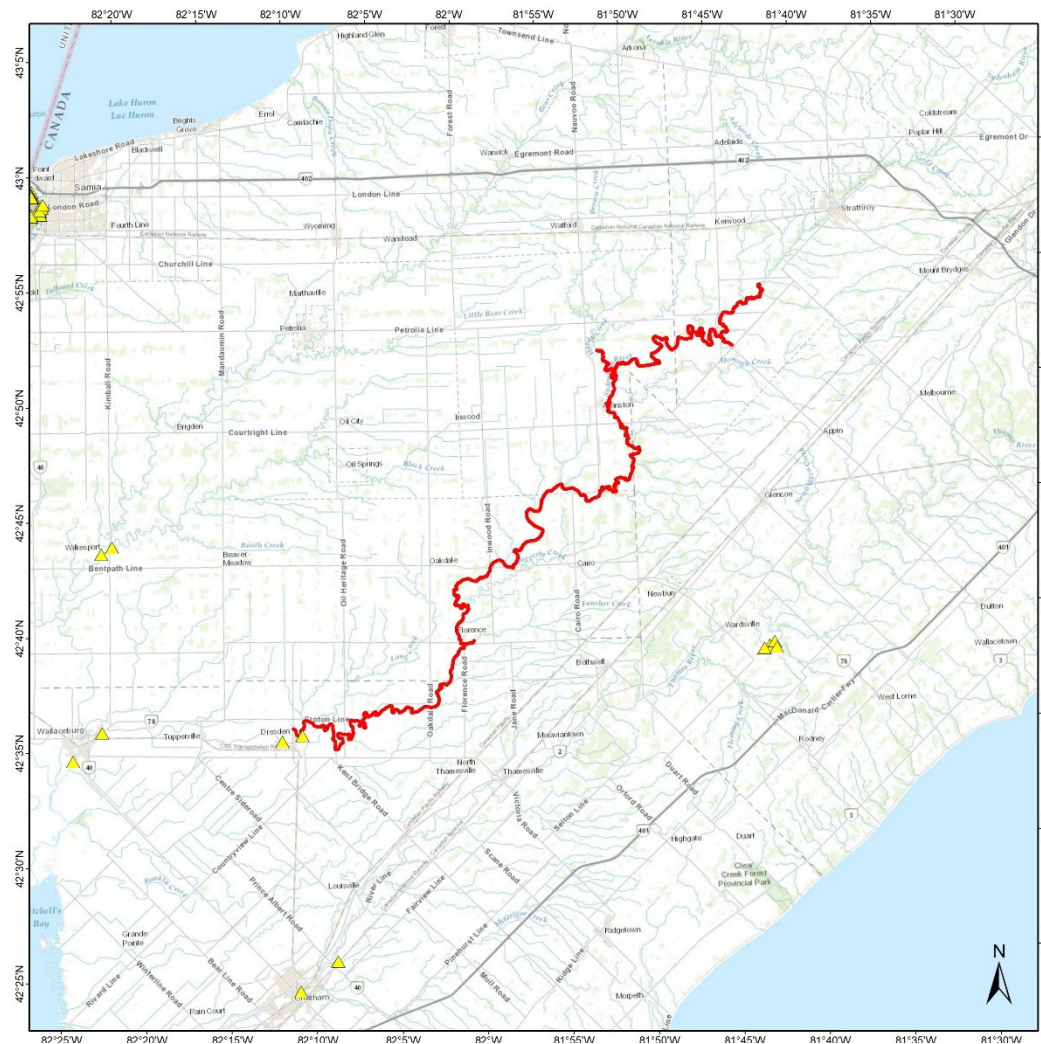


Figure A5.65. Spatial distribution of Round Pigtoe critical habitat (proposed; red lines) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.



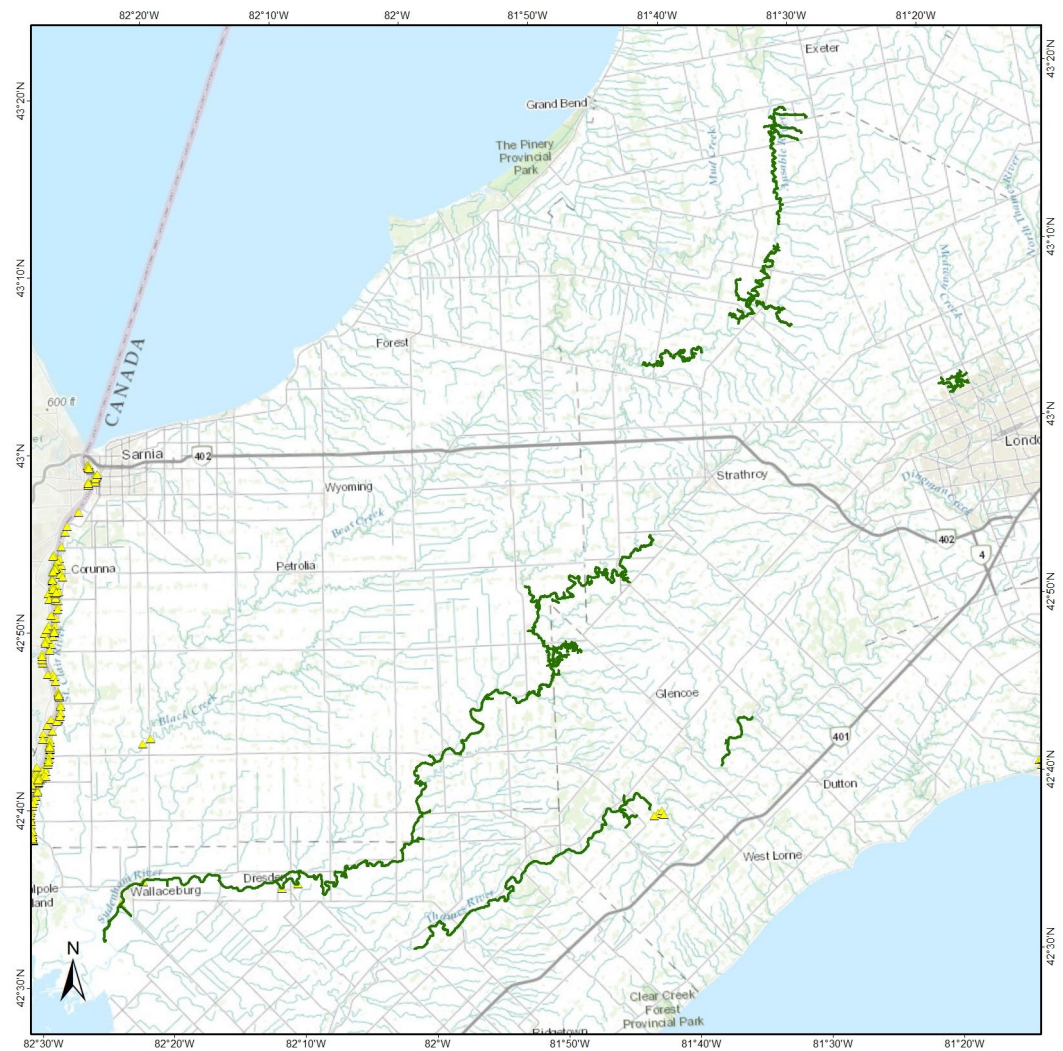


Figure A5.66. Spatial distribution of Kidneyshell (1998–2017; green lines) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.

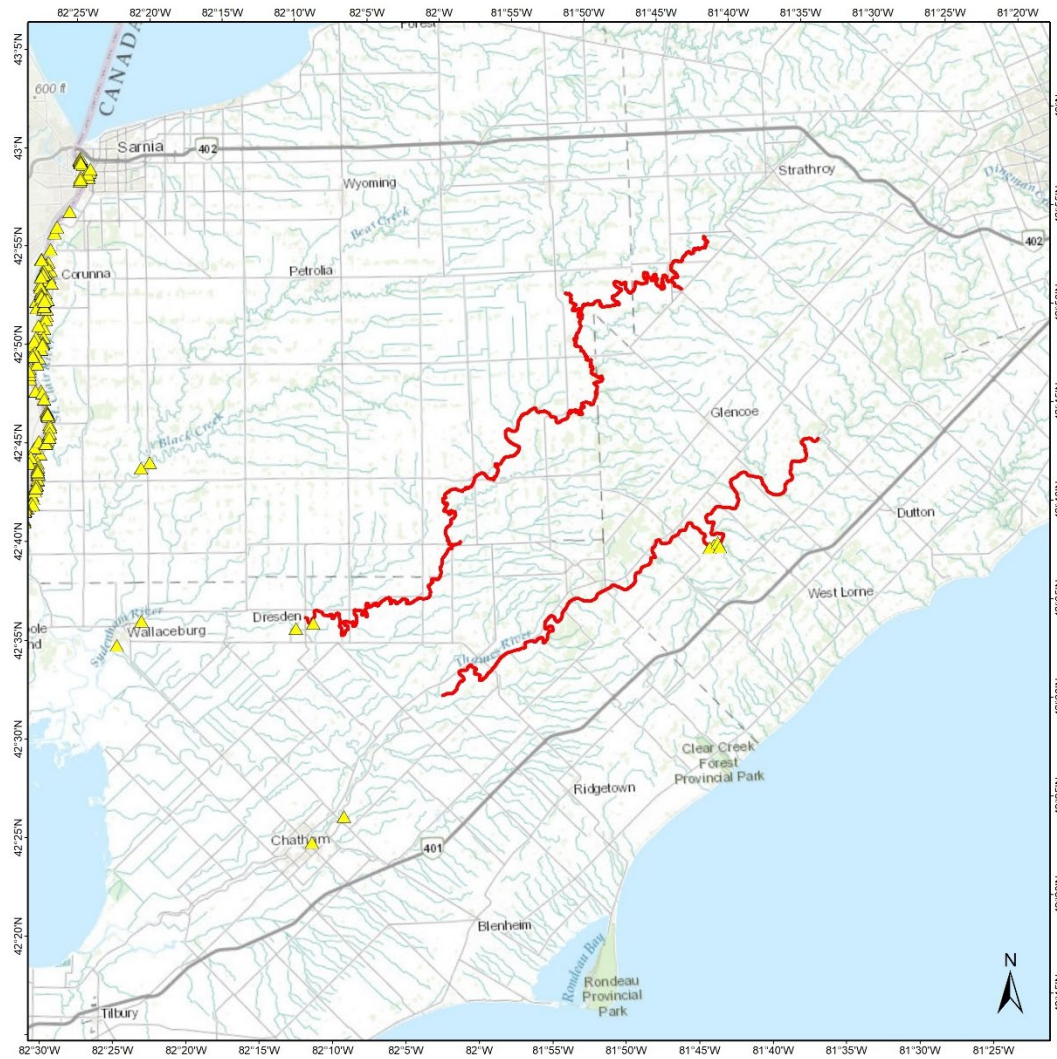


Figure A5.67. Spatial distribution of Kidneyshell critical habitat (red lines) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.



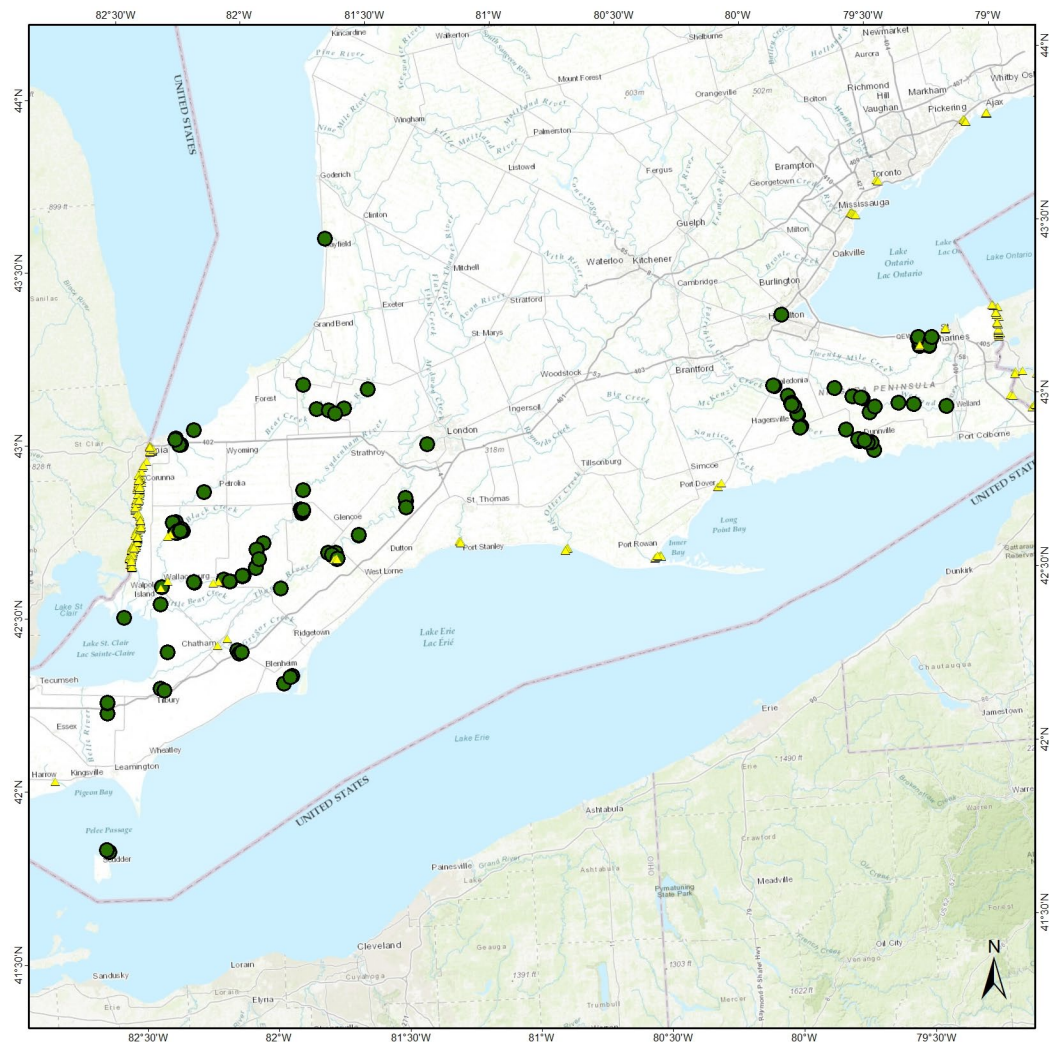


Figure A5.68. Spatial distribution of Mapleleaf records (1998–2017; green circles) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.

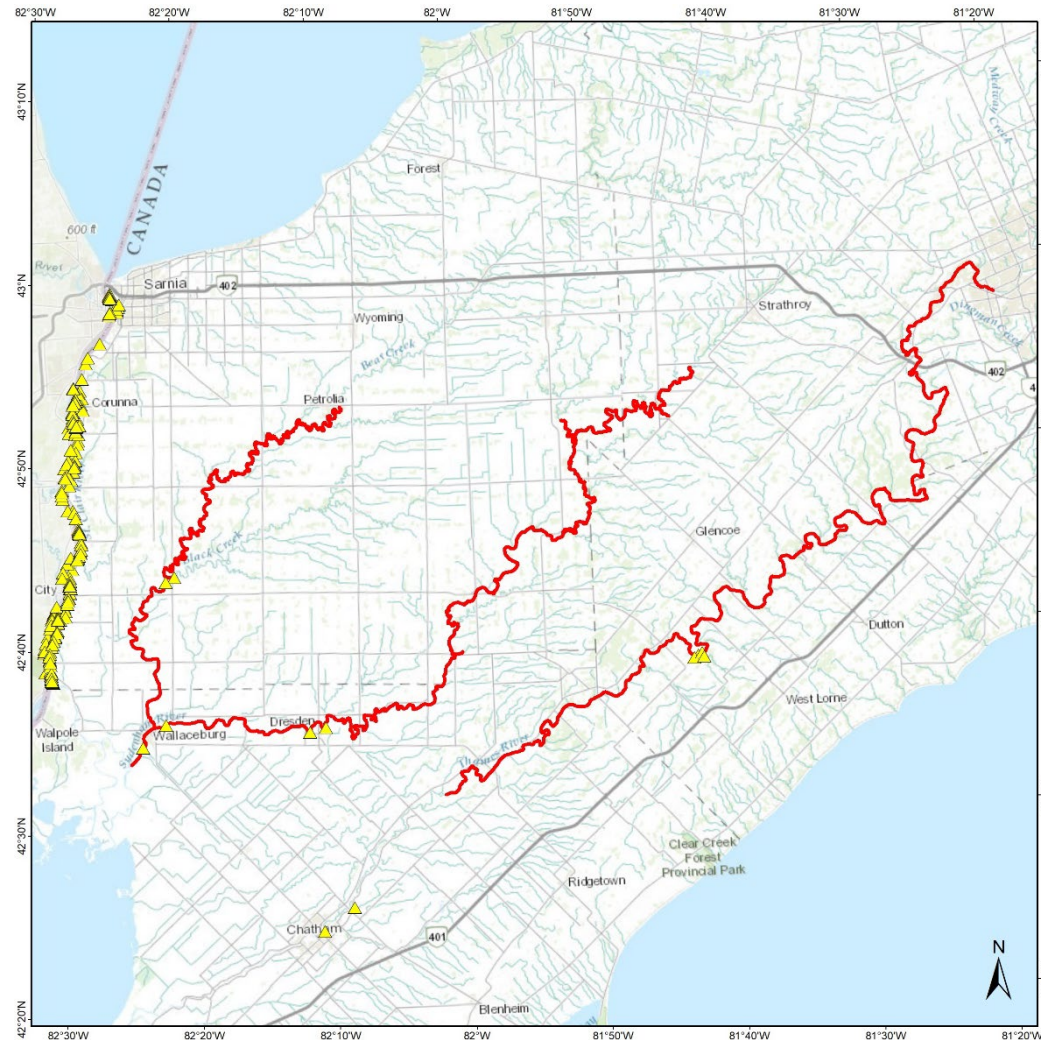


Figure A5.69. Spatial distribution of Mapleleaf critical habitat (proposed; red lines) in the Sydenham and Thames rivers, ON and Bayluscide application locations (2011–2017; yellow triangles). As Mapleleaf was down-listed to Special Concern on Schedule 1 of SARA in August 2019, critical habitat no longer exists for this species.

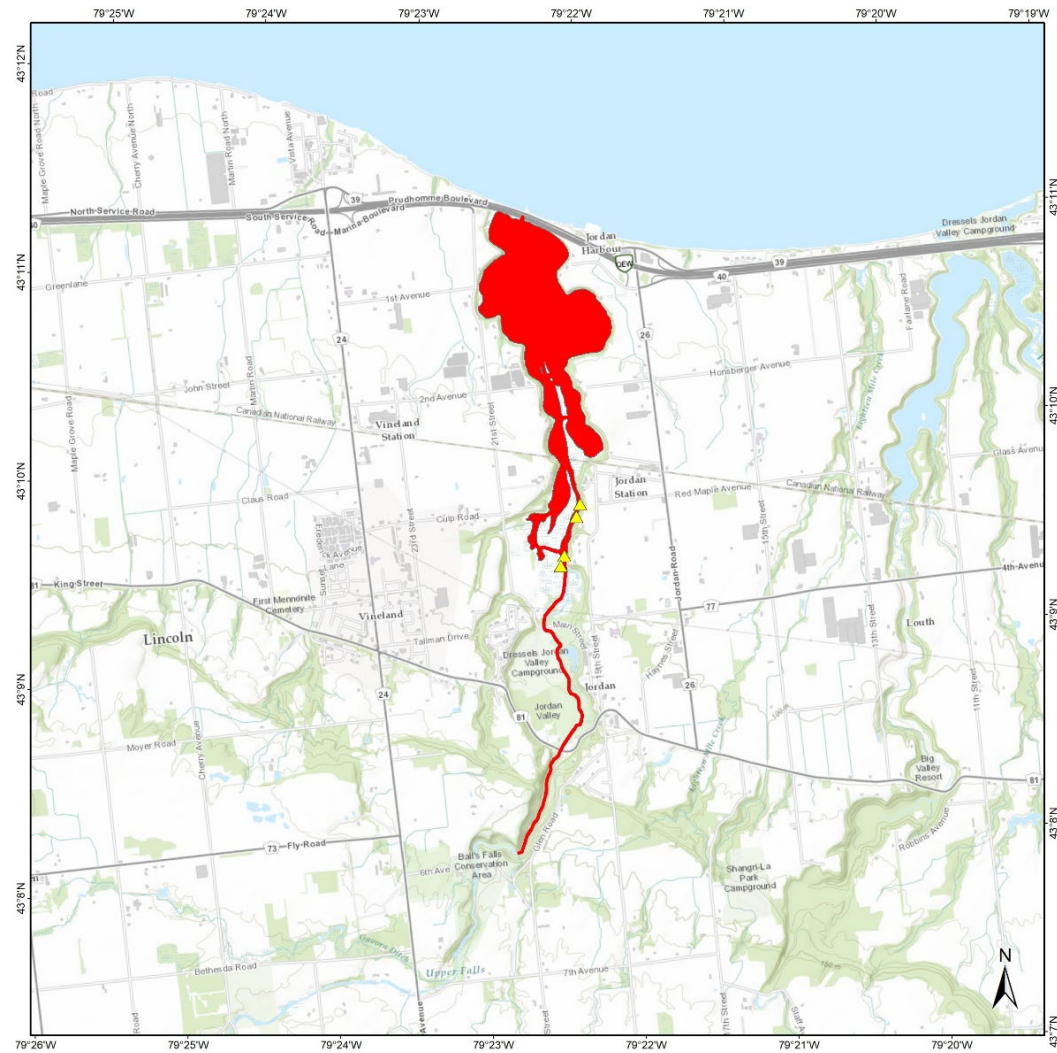


Figure A5.70. Spatial distribution of Mapleleaf critical habitat (proposed; red shaded area) in Jordan Harbour and Twenty Mile Creek, ON and Bayluscide application locations (2011–2017; yellow triangles). As Mapleleaf was down-listed to Special Concern on Schedule 1 of SARA in August 2019, critical habitat no longer exists for this species.





Figure A5.71. Spatial distribution of Salamander Mussel (1998–2017; green lines) and Bayluscide locations (2011–2017; yellow triangles) in Ontario.

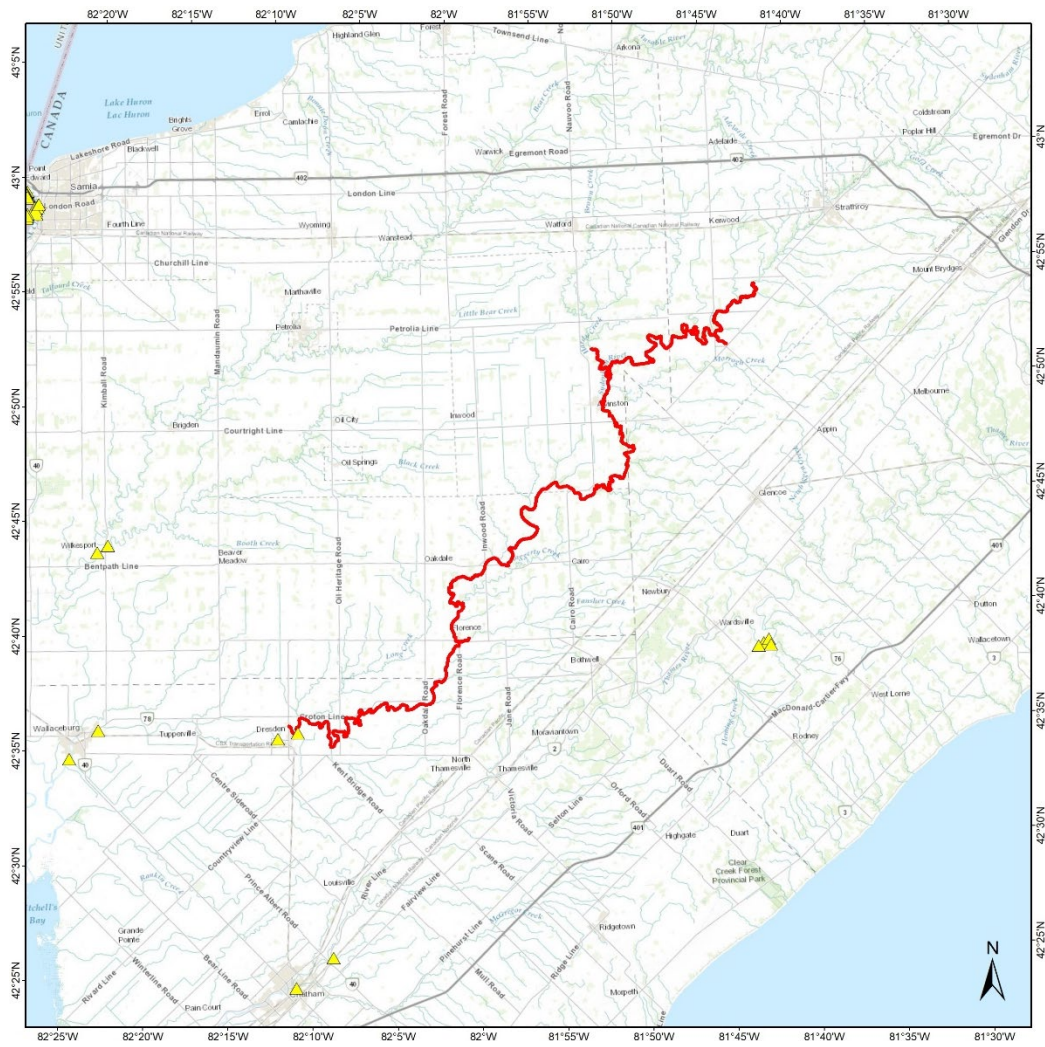


Figure A5.72. Spatial distribution of Salamander Mussel critical habitat (proposed; red lines) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.



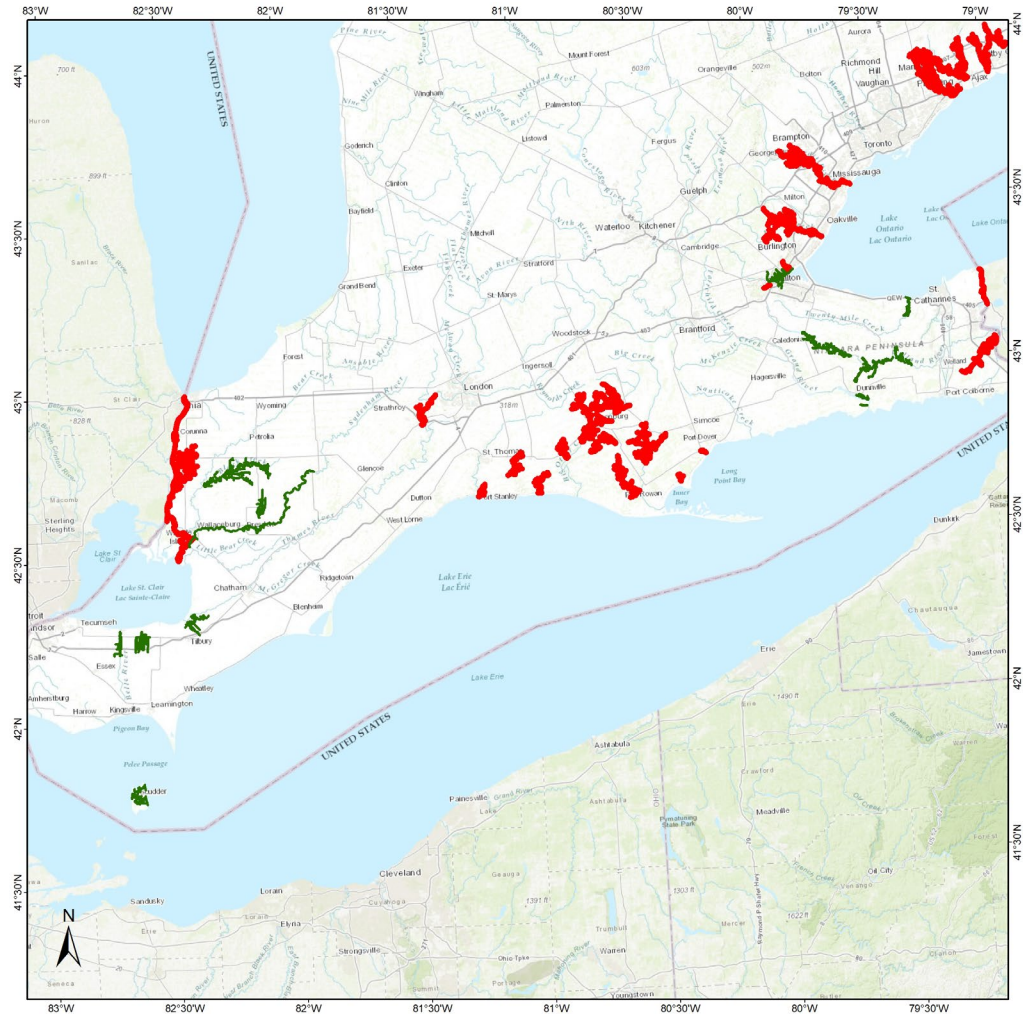


Figure A5.73. Spatial distribution of Lilliput (1998–2017; green shaded area) and larval Sea Lamprey (2011–2017; red shaded area) in Ontario's Great Lakes region.

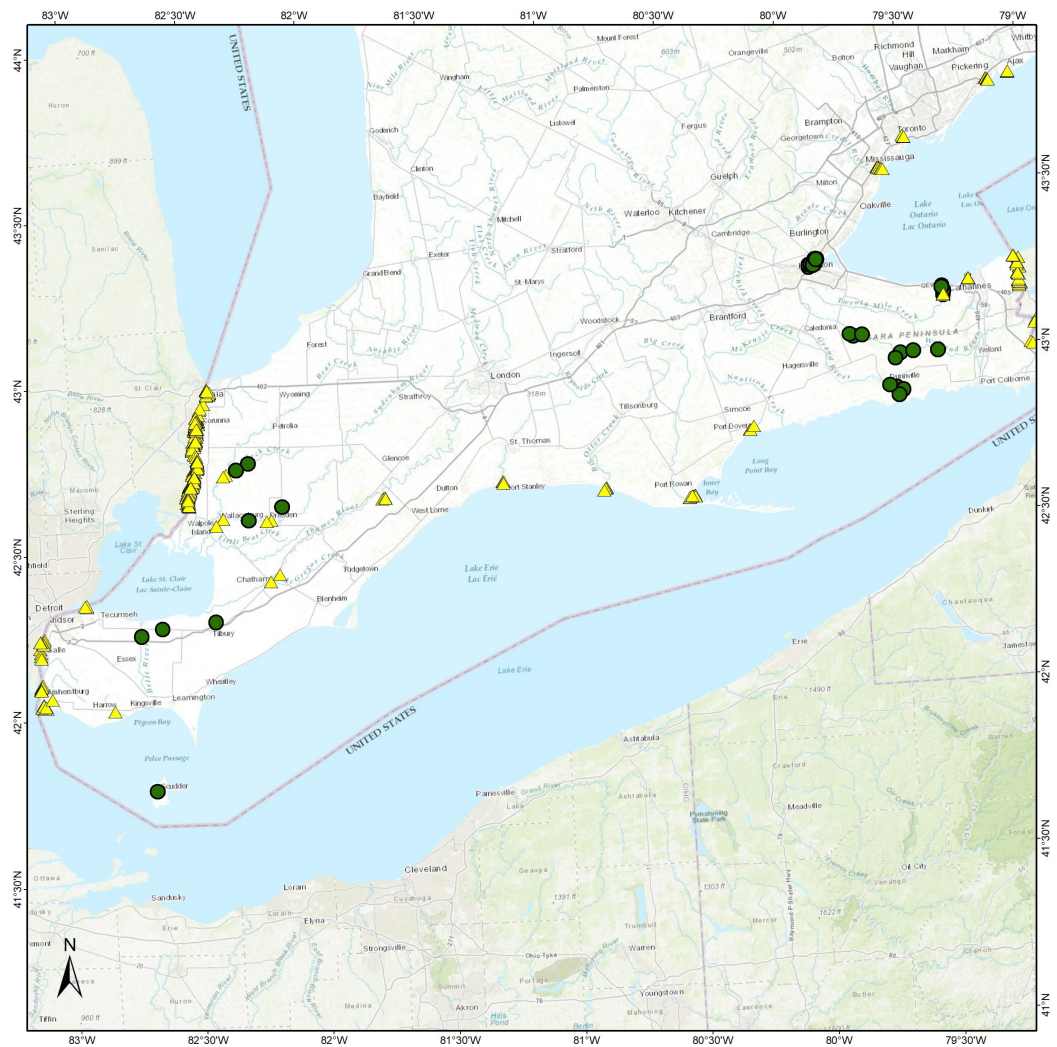


Figure A5.74. Spatial distribution of Lilliput records (1998–2017; green circles) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.

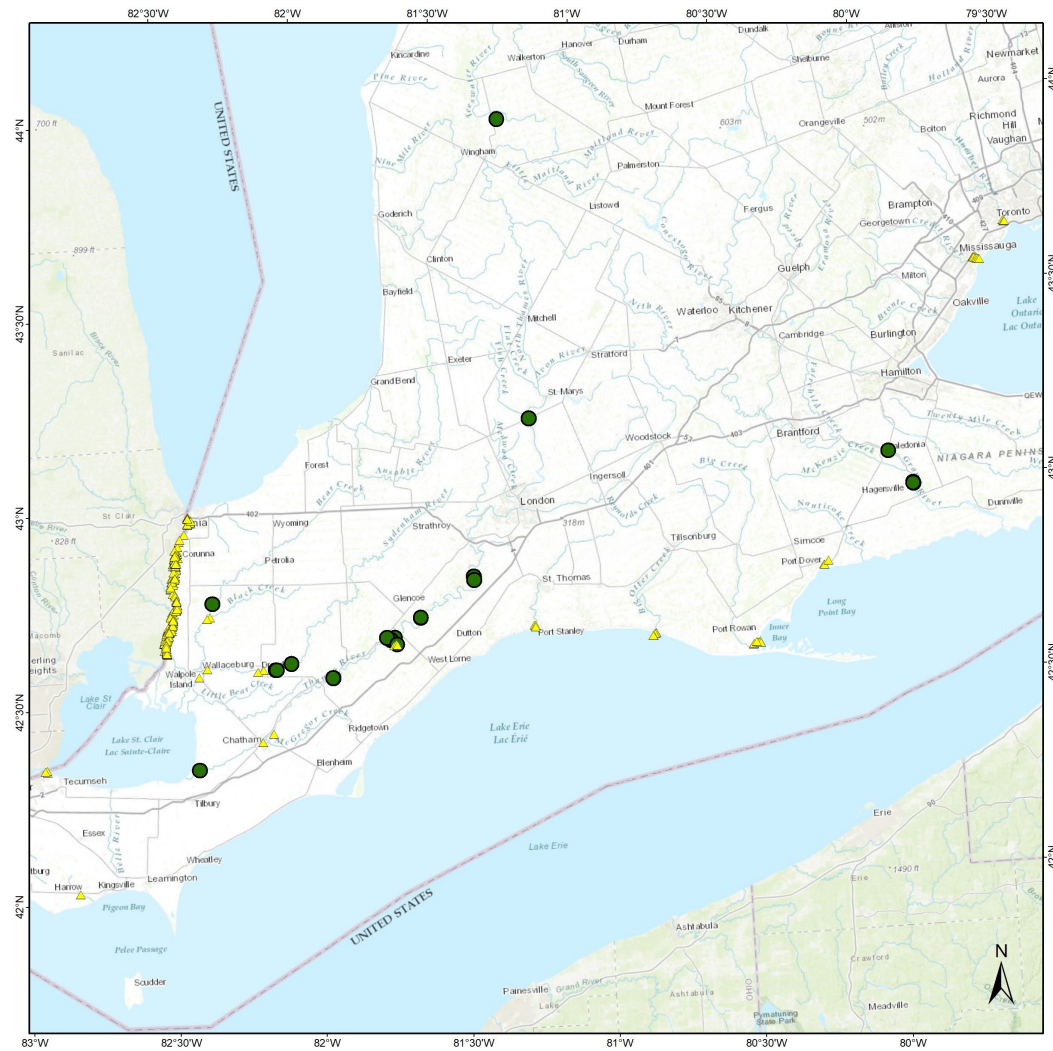


Figure A5.75. Spatial distribution of Fawnsfoot records (1998–2017; green circles) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.



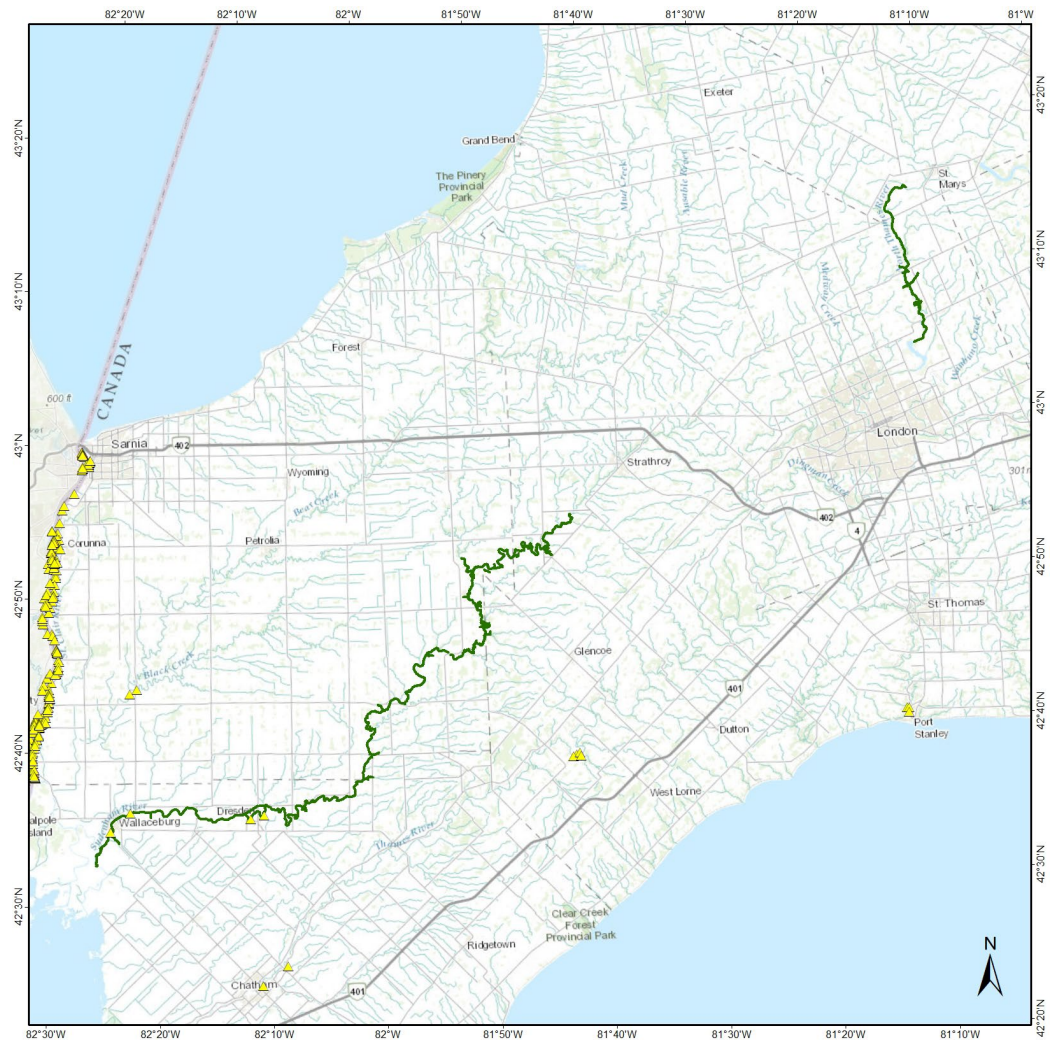


Figure A5.76. Spatial distribution of Rayed Bean records (1998–2017; green lines) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.

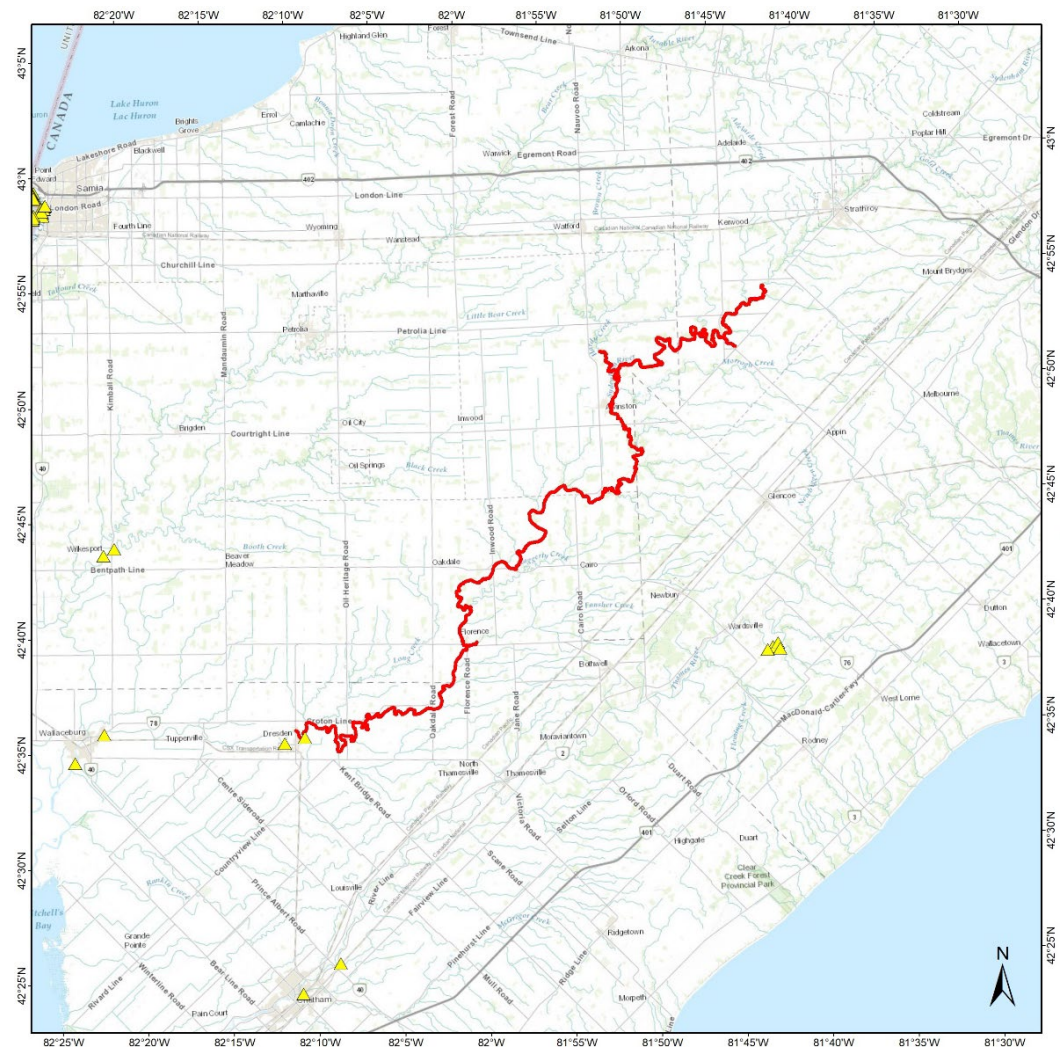


Figure A5.77. Spatial distribution of Rayed Bean critical habitat (proposed; red lines) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.



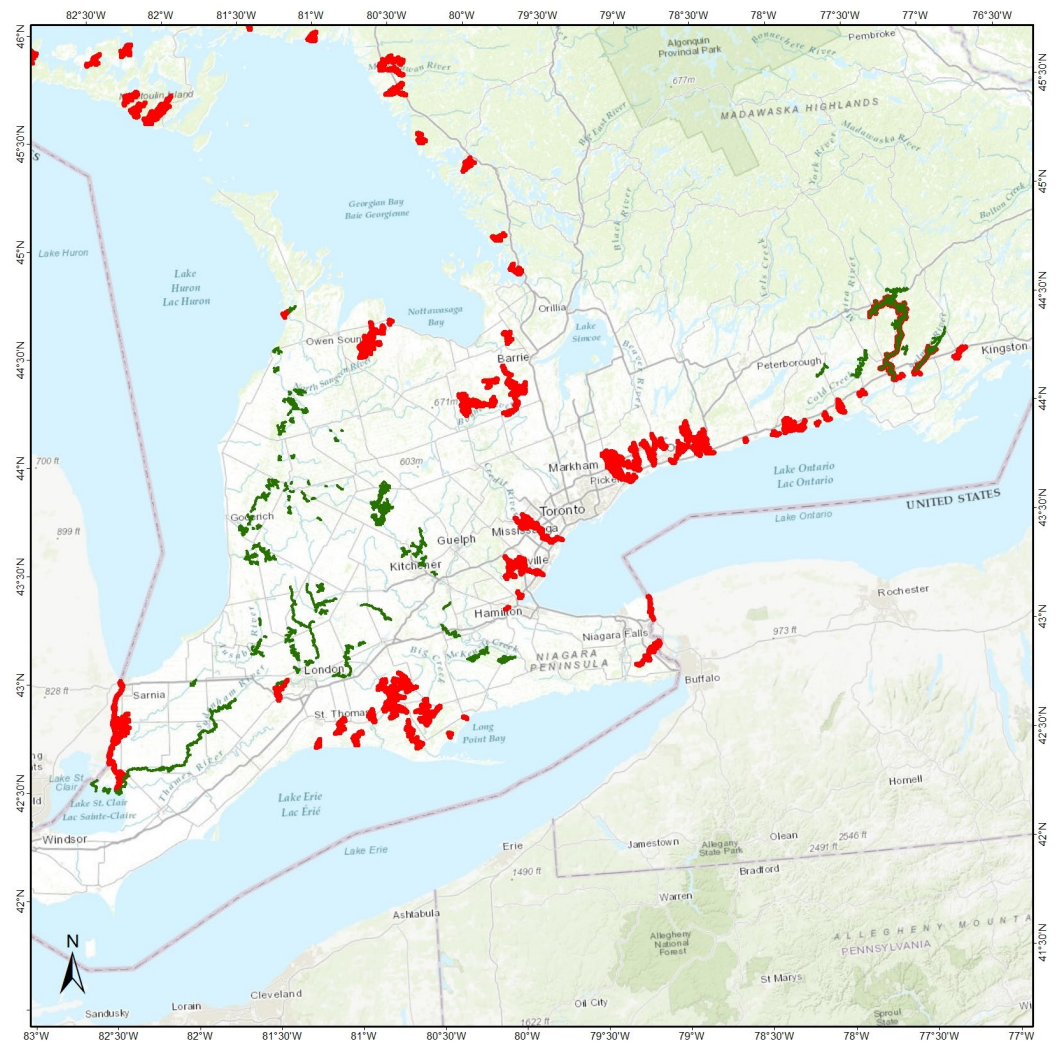


Figure A5.78. Spatial distribution of Rainbow (1998–2017; green shaded area) and larval Sea Lamprey (2011–2017; red shaded area) in Ontario's Great Lakes region.

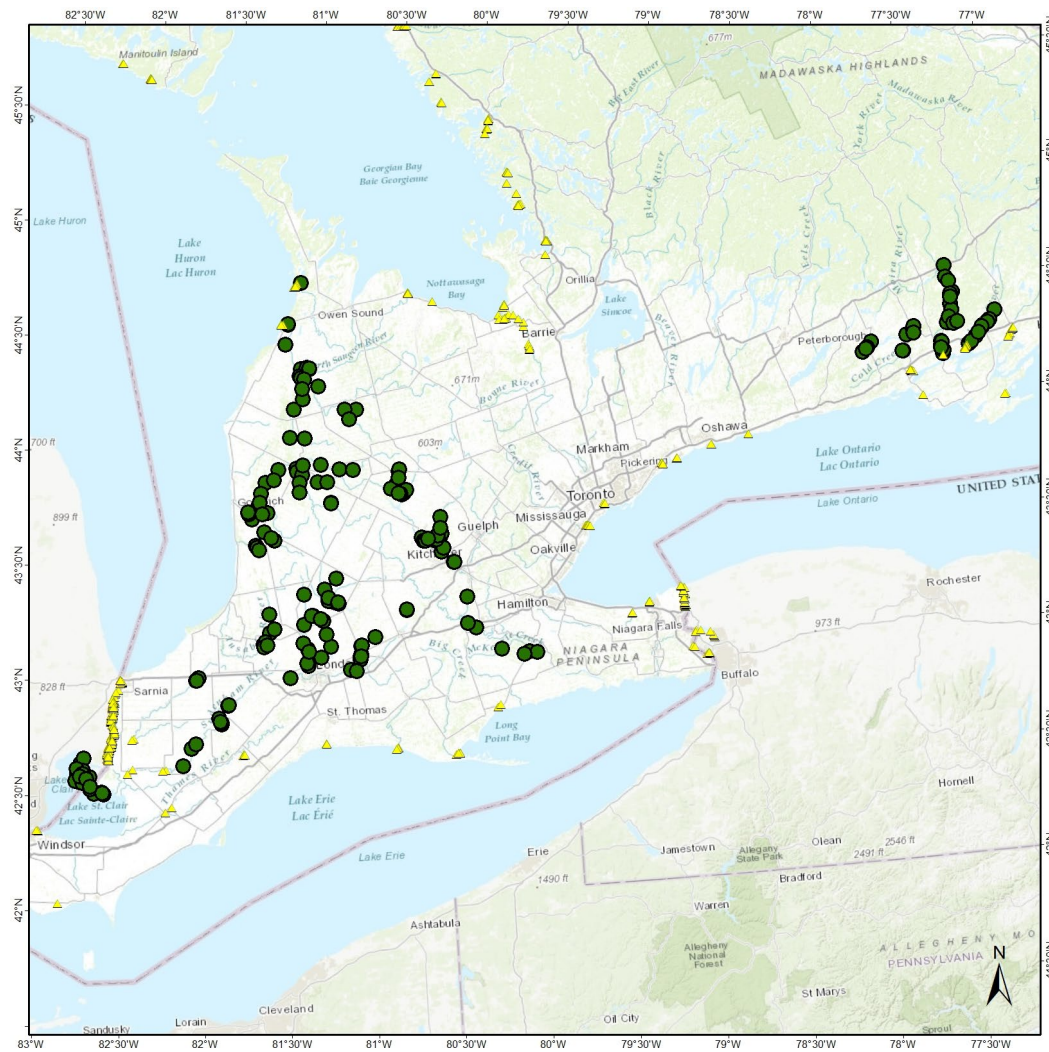


Figure A5.79. Spatial distribution of Rainbow records (1998–2017; green circles) and Bayluscide application locations (2011–2017; yellow triangles) in Ontario.



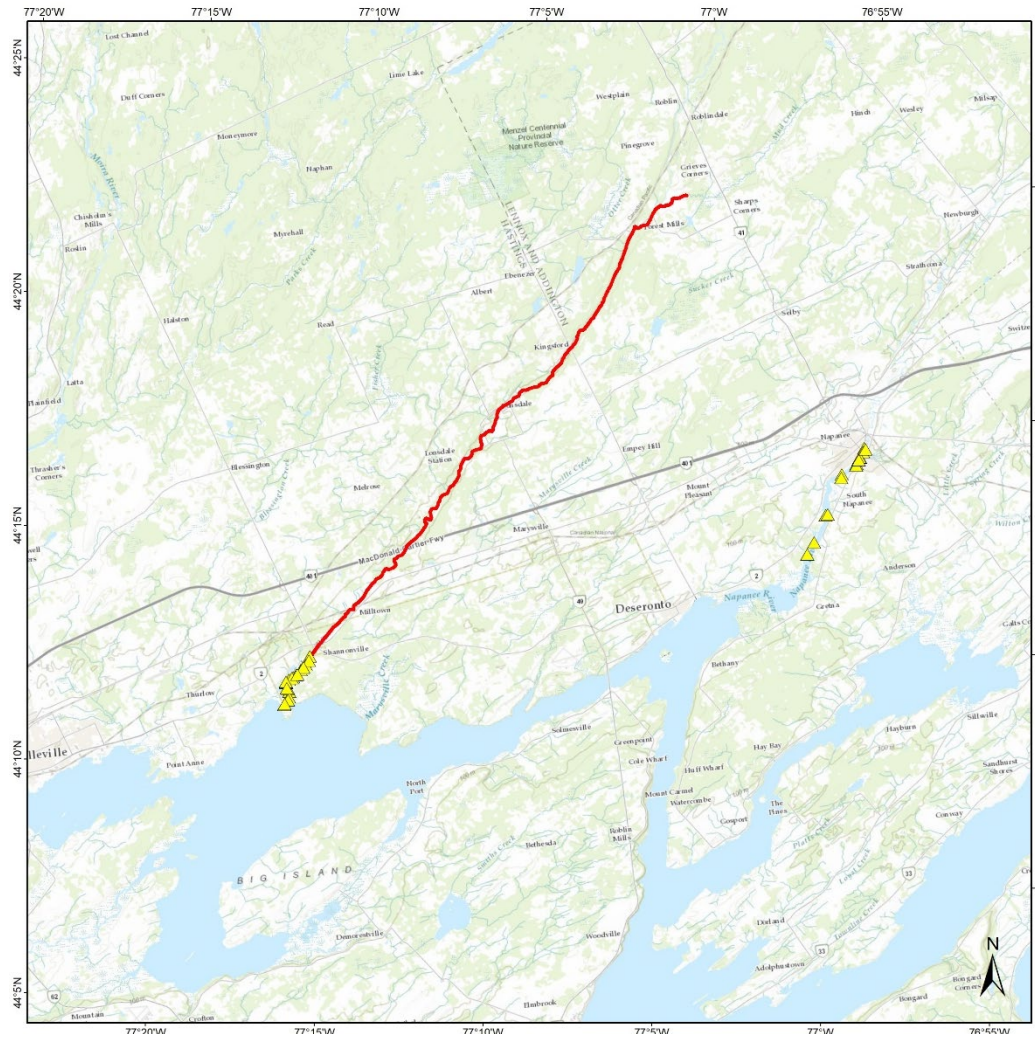


Figure A5.80. Spatial distribution of Rainbow critical habitat (proposed; red line) in the Salmon River, ON and Bayluside application locations (2011–2017; yellow triangles). As Rainbow was down-listed to Special Concern on Schedule 1 of SARA in August 2019, critical habitat no longer exists for this species.