

Reintroduction of fishes in Canada: a review of research progress for SARA-listed species

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Abstract: Fishes are among the most threatened taxa in Canada with over 70 species, subspecies, and (or) designatable units presently listed for protection under the *Species at Risk Act* (SARA). Protecting these species requires a diverse set of strategies based on the best-available data and information. One approach identified under SARA and in Canadian federal recovery strategies for improving the status of SARA-listed fishes is species reintroduction, which involves the release of individuals into areas from which they have been extirpated with the goal of re-establishing self-sustaining populations. The success of reintroduction relies on a comprehensive understanding of species ecology and life history, with considerations around population genetics and genomics. However, SARA-listed species are some of the most poorly known species in Canada due to their rarity and relative lack of research investment prior to the enactment of SARA. As a result, SARA-listed species have the most to lose if reintroduction activities are not carefully researched, planned, and executed. Therefore, the purpose of this review is to present an accessible summary on the state of reintroduction science for SARA-listed fishes in Canada with the hope of motivating future research to support reintroduction activities. We focus our review on 14 SARA-listed freshwater or anadromous fishes identified as candidates for reintroduction in federal recovery strategies. We follow the species-specific summaries with guidance on how basic research questions in population ecology, habitat science, and threat science provide a critical foundation for addressing knowledge gaps in reintroduction science. Subsequently, we identify the importance of genetic and genomic techniques for informing future research on the reintroduction of SARA-listed species. We conclude with recommendations for active, experimental approaches for moving reintroduction efforts forward to recover Canadian fishes.

Key words: Canada, conservation, endangered, freshwater fish, species at risk.

Résumé : Les poissons comptent parmi les taxons les plus menacés au Canada, avec plus de 70 espèces, sous-espèces et/ou unités désignables actuellement inscrites à la liste des espèces à protéger en vertu de la *Loi sur les espèces en péril* (LEP). La protection de ces espèces nécessite un ensemble diversifié de stratégies établi en fonction des meilleures données et informations disponibles. La réintroduction d'espèces est une approche définie dans la LEP et dans les programmes de rétablissement fédéraux canadiens pour améliorer la situation des poissons inscrits à la LEP, ce qui implique l'introductions d'individus dans des zones d'où ils ont été extirpés dans le but de rétablir des populations capables de s'autoperpétuer. Le succès de la réintroduction repose sur une compréhension globale de l'écologie des espèces et de l'évolution biologique, avec des considérations relatives à la génétique et à la génomique des populations. Toutefois, les espèces inscrites sur la liste de la LEP figurent parmi les espèces les moins connues au Canada en raison de leur rareté et du manque relatif d'investissements en recherche avant l'adoption de la LEP. Ainsi, les espèces inscrites à la LEP ont le plus à perdre si les activités de réintroduction ne sont pas soigneusement étudiées, planifiées et mises en œuvre. Par conséquent, le présent document vise à présenter un résumé accessible de l'état de la science de la réintroduction des poissons inscrits à la LEP au Canada dans l'espoir de motiver les recherches futures à l'appui des activités de réintroduction. Notre examen porte sur 14 poissons d'eau douce ou anadromes inscrits sur la liste de la LEP considérés comme des candidats pour la réintroduction dans les programmes fédéraux de rétablissement. Nous suivons les résumés spécifiques de ces espèces avec des conseils sur la façon dont les questions de recherche fondamentale sur l'écologie des populations, la science des habitats et la science des menaces fournissent une base essentielle pour combler les lacunes dans les connaissances scientifiques sur la réintroduction. Par la suite, nous avons cerné l'importance des techniques génétiques et génomiques afin d'éclairer les futures recherches futures sur la réintroduction des espèces inscrites à la LEP. Nous concluons en recommandant des approches actives et expérimentales pour faire avancer les efforts de réintroduction afin de rétablir les poissons canadiens. [Traduit par la Rédaction]

Mots-clés : Canada, conservation, en voie de disparition, poissons d'eau douce, espèces en péril.

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Introduction

Anthropogenic stressors such as habitat alteration, invasive species, and other landscape stressors, combined with changing thermal conditions due to climate change, are transforming global fish species distributions (Comte et al. 2013) and abundance (Baillie et al. 2010). Threats to aquatic biodiversity are often realized as species losses, prompting most developed countries to enact legislation to protect and recover species at risk of extinction. In Canada, there are over 70 species, subspecies, and (or) designatable units (DUs; COSEWIC 2015) of fishes presently listed for protection under Schedule 1 of the federal *Species at Risk Act* (SARA; SARA 2002). DUs are populations or groups of populations that exhibit geographic or genetic distinctiveness and evolutionary significance (COSEWIC 2017). SARA is designed to protect and recover imperilled species listed as “Threatened”, “Endangered”, or “Extirpated”. Federal recovery strategies for these species provide recommendations on how to protect and recover each species based on descriptions of species-specific information including life history, threats, and habitat requirements. In some cases, conservation and recovery approaches may be presented in an ecosystem framework, with objectives and approaches for protecting multiple species simultaneously (Poos et al. 2008). Examples of conservation and recovery recommendations include identification and protection of critical habitat (a legal requirement within the Act), minimizing threats to species and their habitat, and supplementing populations or initiating reintroduction efforts.

Reintroduction efforts involve releasing fishes into the wild to re-establish populations in areas where the species has been lost. Reintroduction can be performed by removing individuals from one population to re-establish a population in a formerly occupied location (i.e., translocation; Galloway et al. 2016) or by means of captive-breeding efforts, which typically involve a greater degree of research effort and supporting infrastructure (e.g., dedicated hatchery facilities). In comparison to terrestrial species, reintroduction of fishes is relatively rare (Seddon et al. 2007) and our understanding of what makes reintroduction efforts successful is limited (Cochran-Biederman et al. 2015). For example, despite comprising more than half of all described vertebrate species, only about 4% of published repatriation efforts include fishes (Seddon et al. 2005) and, of 263 cases of reintroducing vertebrates, approximately 40% focused on birds, 27% on mammals, and 24% on fishes (Champagnon et al. 2012). However, given the continued threats to aquatic ecosystems and ongoing species decline, reintroduction will likely become an increasingly important strategy to ensure the recovery of fishes in Canada.

Initiating species reintroduction requires knowledge of the life history and ecology of imperilled species to allow evidence-based hypotheses and management expectations to be assessed (e.g., minimum viable population size required for successful releases). As well, a thorough understanding of the ecosystem conditions where the species has been extirpated is needed to avoid stocking individuals into areas of potentially unsuitable habitats. However, the biology and ecology of most fishes listed under SARA are poorly known, with many Canadian populations being understudied from a research and monitoring perspective compared to commercially or recreationally important species. For example, SARA-listed species often lack information commonly known for commercial species that is necessary for basic management decisions such as fecundity, age structure, or mortality rates. As well, populations of SARA-listed species are typically sparse, present unique sampling challenges (e.g., nonlethal sampling), and often lack intensive population monitoring programs, making many aspects of reintroduction science difficult (e.g., identification of suitable source populations for translocation, among others). These issues, combined with dedicated research on SARA-listed species having intensified only since the inception of SARA (2002),

have meant that advanced questions for reintroduction, like effective stocking densities to achieve population persistence, have yet to be addressed for most species in Canada. Where dedicated research successes have occurred, research has often focused on identifying critical habitat and threats (e.g., Poos et al. 2012), with science to support species reintroductions lagging (Lamothe and Drake 2019).

The purpose of this review is to present an accessible summary of the existing knowledge and reintroduction progress for all SARA-listed freshwater and anadromous fishes in Canada that are candidates for, or have undergone, reintroduction efforts. By reviewing the current state of species reintroductions under SARA, our goals were twofold: (i) to identify species having the greatest elements of success that could guide future reintroduction programs for other species and (ii) to identify species-specific gaps to motivate future research that will have the greatest benefit to SARA-listed species in Canada. We provide baseline information for freshwater or anadromous species identified in federal recovery strategies as candidates for reintroduction efforts including Atlantic Salmon (*Salmo salar*), Atlantic Whitefish (*Coregonus huntsmani*), Channel Darter (*Percina copelandi*), Copper Redhorse (*Moxostoma hubbsi*), Eastern Sand Darter (*Ammocrypta pellucida*), Gravel Chub (*Erimystax x-punctatus*), Lake Chubsucker (*Erimyzon sucetta*), Northern Madtom (*Noturus stigmosus*), Pugnose Shiner (*Notropis anogenus*), Redside Dace (*Clinostomus elongatus*), Spring Cisco (*Coregonus* sp.), Striped Bass (*Morone saxatilis*), Westslope Cutthroat Trout (*Oncorhynchus clarkii lewisi*), and White Sturgeon (*Acipenser transmontanus*; Table 1; Fig. 1). Although many other SARA-listed species need increased research to achieve recovery, we focused on species with reintroductions explicitly identified as a priority in federal recovery strategies. Following these species-specific summaries, we describe how research theme areas involving population ecology, habitat science, and threat science can improve progress of reintroduction efforts for fishes in Canada, and we identify genetic and genomic factors when considering reintroduction initiatives. Finally, we conclude by advocating for an adaptive approach to reintroductions in Canada to ensure informative and timely recovery actions for fishes at risk of extirpation.

Atlantic Salmon—Inner Bay of Fundy DU

Atlantic Salmon is a well-studied, large-bodied (adult average total length (TL) = 60 cm; Fisheries and Oceans Canada 2010a), typically anadromous salmonid that spends most of its adult life in the ocean, but returns to natal rivers to spawn. This homing behaviour results in barriers to gene flow and subsequent reproductive isolation, leading to distinct populations across the species range that has resulted in the identification of several intraspecific DUs. The inner Bay of Fundy (iBoF) DU was first listed under SARA as Endangered in 2003 and includes Atlantic Salmon from all rivers that drain into the Bay of Fundy, from the Mispic River in New Brunswick to the Pereaux River in Nova Scotia (Fisheries and Oceans Canada 2010a). The iBoF DU is morphologically indistinguishable from other Atlantic Salmon populations, but it differs genetically (Verspoor et al. 2002) and in life-history characteristics (e.g., shorter migration, earlier age at maturity; Fisheries and Oceans Canada 2010a). In the early 20th century, the iBoF DU consisted of more than an estimated 40 000 adults, but that number has plummeted in present times to fewer than 300 adults (COSEWIC 2006; Fisheries and Oceans Canada 2010a). Extirpations have occurred in several rivers including the Point Wolfe, Upper Salmon, and Petitcodiac rivers (Fraser et al. 2007; Fisheries and Oceans Canada 2016a).

Given the typical anadromous life history of Atlantic Salmon, threats occur in both freshwater and marine environments. During the freshwater life stages, Atlantic Salmon prefer clean, cool (summer temperatures 15–25 °C), and well-oxygenated waters, with pH levels >5.5, low to moderately steep gradients, and

Table 1. Reintroduction status of SARA-listed species in Canada where reintroduction was listed as a recovery approach in federal species recovery strategies.

Designatable unit	Status		Reintroduction goal	Has reintroduction* occurred?	Status of reintroduction program
	COSEWIC	SARA			
Atlantic Salmon (Inner Bay of Fundy)	EN	EN	“To re-establish wild, self-sustaining populations as required to conserve the genetic characteristics of the remaining anadromous iBoF Atlantic salmon” (p. 2; Fisheries and Oceans Canada 2010a).	Yes	Live Gene Bank stocking program has been ongoing since 1998
Atlantic Whitefish	EN	EN	“To achieve stability in the current population of Atlantic Whitefish in Nova Scotia, reestablishment of the anadromous form, and expansion beyond its current range” (p. viii; Fisheries and Oceans Canada 2018b).	Yes	Captive breeding program facility decommissioned in 2015
Channel Darter	—	TH	“To investigate the feasibility of various re-establishment approaches for Channel Darter and identify appropriate source populations,” and to “determine if there are extirpated or new sites that are suitable for threat mitigation or habitat restoration for potential re-establishment” (p. 25; Fisheries and Oceans Canada 2013).	No	NA
Copper Redhorse	EN	EN	“To attain a population of 4000 mature individuals over a period of 20 years while “support[ing] the Copper Redhorse population through stocking until natural reproduction can ensure the long-term stability of the population” (p. iii; Fisheries and Oceans Canada 2012a).	Yes	Captive breeding and population supplementation have been ongoing since 2004
Eastern Sand Darter (Ontario)	TH	TH	“To investigate the feasibility of population supplementation or repatriation for populations that may be extirpated or reduced” (p. iv; Fisheries and Oceans Canada 2012b).	No	NA
Gravel Chub	EX	EX	“To examine the feasibility of relocations, captive-rearing, and re-introductions” (p. v; Edwards et al. 2007).	No	NA
Lake Chubsucker	EN	EN	“To determine the feasibility of repatriation for populations that may be extirpated or reduced” (p. iii; Staton et al. 2010).	No	NA
Northern Madtom	EN	EN	“To determine the feasibility of relocations and captive rearing” (p. v; Edwards et al. 2012).	No	NA
Pugnose Shiner	TH	EN	“To investigate the feasibility of population supplementation or repatriation for populations that may be extirpated or reduced” (p. vii; Fisheries and Oceans Canada 2012c).	No	NA
Redside Dace	EN	EN	“To restore viable populations of Redside Dace in a significant portion of its historical range in Ontario by: (i) protecting existing healthy, self-sustaining populations and their habitats; (ii) restoring degraded populations and habitats; and, (iii) re-establish Redside Dace to sites of former distribution, where feasible” (p. 12; Fisheries and Oceans Canada , unpublished data).	No	NA
Spring Cisco	EN	EN	“To stock Lac des Écorces with spring ciscoes” by “develop[ing] a reproduction and growth plan, developing a reproduction method for fish farming” (p. 13; Fisheries and Oceans Canada 2014b).	No	NA
Striped Bass (St. Lawrence River)	EN	EX	“To increase the present number of Striped Bass in the St. Lawrence by stocking 50 000 autumn fry each year” (p. 23; Robitaille et al. 2011).	Yes	Reintroduction activities suspended due to signs of recovery (broodstock will be maintained)
Westslope Cutthroat Trout (Alberta)	TH	TH	“To protect and maintain the existing ≥ 0.99 pure populations at self-sustaining levels, and re-establish additional pure populations to self-sustaining levels, within the species’ original distribution in Alberta” (p. iii; Fisheries and Oceans Canada 2014c).	No	NA
White Sturgeon	EN and TH [†]	NA [‡]	“Population supplementation is proposed as a temporary, but long term (potentially 40+ years) measure to prevent extirpation of impacted” white sturgeon populations (p. 46; Fisheries and Oceans Canada 2014d).	Yes	Captive breeding and population supplementation have been ongoing since 1990

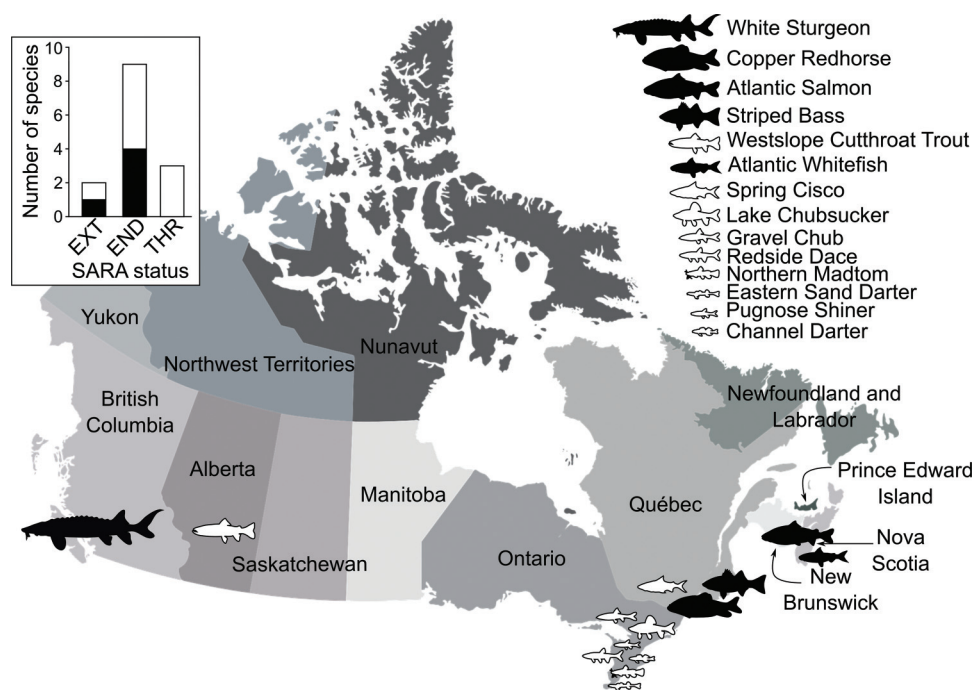
Note: SARA, *Species at Risk Act*; COSEWIC, Committee on the Status of Endangered Wildlife in Canada; EN, endangered; iBoF, inner Bay of Fundy; TH, threatened; NA, not applicable; EX, extirpated.

*Reintroduction as used here includes population supplementation, translocation, managed relocation for the purposes of species recovery, or release of captive-bred individuals.

[†]EN in Upper Kootenay, Upper Columbia, Upper Fraser; TH in Lower Fraser.

[‡]Listing of White Sturgeon designatable units (DUs) are being considered under the new COSEWIC DU structure.

Fig. 1. Species at Risk Act (SARA)-listed fishes identified as candidates for reintroduction in federal recovery strategies. Black species and bars have had some aspects of reintroduction initiated (includes captive breeding, supplementation, or translocation). White species and bars have not had reintroduction initiated. Inset plot: number of species with reintroduction efforts initiated based on SARA status. Note: Westslope Cutthroat Trout is stocked for recreational angling in Alberta, not for conservation, so it is not included as reintroduction for conservation. EXT, Extirpated; END, Endangered; THR, Threatened.



substrates composed of gravel, cobble, and boulder (Elson 1975; Fisheries and Oceans Canada 2010a). Present-day conditions, although not pristine, suggest that freshwater habitats are not limiting iBoF populations and that population viability cannot be improved through increased freshwater habitat (Trzcinski et al. 2004); in fact, there are indications that juvenile abundance has increased in rivers as a result of management actions (Live Gene Banking described below; Fisheries and Oceans Canada 2008). Instead, declines in iBoF Atlantic Salmon abundance are thought to be due to high mortality rates at sea (Amiro and Jefferson 1996) caused by the cumulative effects of stressors (e.g., interactions with farmed and hatchery salmon, fisheries) leading to the deterioration of marine habitat quality (Fisheries and Oceans Canada 2008; COSEWIC 2010a). Specifically, tidal barriers, aquaculture, primary production declines, climate change, among other factors have been identified as contributing to poor marine survival (COSEWIC 2010a); however, their effects on Atlantic Salmon survival and distribution have not been well quantified due to the challenges of studying this species across such large spatial and temporal scales.

Despite the threats facing Atlantic Salmon and unique life-history characteristics, Atlantic Salmon biology is one of the best studied among SARA-listed fishes, much of that due to the early success of captive breeding, rearing, and stocking efforts. Between 1900 and 2003, over 40 million Atlantic Salmon have been released into iBoF rivers (Gibson et al. 2003). Formal captive-breeding for the iBoF Atlantic Salmon began in 1998 with the Live Gene Bank program (O'Reilly and Kozfkay 2014). Fisheries and Oceans Canada maintains the Live Gene Bank program at the Mactaquac and Coldbrook biodiversity facilities (Fisheries and Oceans Canada 2010a). The Live Gene Bank program is currently focused on four rivers (Stewiacke, Gaspereau, Big Salmon, and Point Wolfe rivers; Fisheries and Oceans Canada 2016a), but Atlantic Salmon have also been released into Weldon Creek and the Upper Salmon, Demoiselle, Petitcodiac, Black, Economy, Great

Village, Debert, Folly, Salmon, Cornwallis, and Portapique rivers (Fisheries and Oceans Canada 2016a). The primary emphases for iBoF Atlantic Salmon captive-breeding efforts are to minimize the loss of genetic variation and to reduce the rates of adaptation to captivity (O'Reilly and Kozfkay 2014) as differences in aerobic capacity, aggressiveness, genetic diversity, and growth have been demonstrated between captive-reared and wild Atlantic Salmon (McDonald et al. 1998; Blanchet et al. 2008; Bowlby and Gibson 2011; Wilke et al. 2015). A strategy for minimizing the ongoing loss of genetic diversity and adaptations to captivity is to breed iBoF Atlantic Salmon that have been exposed to wild-river conditions as juveniles (Fisheries and Oceans Canada 2016a, 2018a).

Without the Live Gene Bank program, Atlantic Salmon in the iBoF would likely be extinct (Fisheries and Oceans Canada 2008, 2018a); therefore, the continued existence of the iBoF Atlantic Salmon is dependent on this program. However, greater emphasis is needed to understand the causes of decline of iBoF salmon, particularly during the marine transition from smolt to adult (Bowlby and Gibson 2015). A recent review of the science associated with the Live Gene Bank and supplementation programs for iBoF Atlantic Salmon indicated that the number of adults returning to their native rivers per year is between 0–10 individuals (Fisheries and Oceans Canada 2018a). Acoustic-tracking studies on smolts are ongoing to better understand smolt marine-habitat preference and, consequently, relate potential threats to smolt behaviour (Fisheries and Oceans Canada 2016a). As well, efforts are underway to understand the impacts of disease and parasite load on iBoF Atlantic Salmon, to improve monitoring and management of sea lice, and to restore connectivity in the Bay of Fundy (Fisheries and Oceans Canada 2016a).

Atlantic Whitefish

Atlantic Whitefish is a moderate-sized (adult average TL = 20–40 cm; Bradford et al. 2010), cool-water salmonid with silver sides

and a silver to white underbelly endemic to Nova Scotia (Fisheries and Oceans Canada 2018b). The Tusket and Annis rivers, which share a common estuary in Yarmouth County, and the Petite Rivière, Lunenburg County, Nova Scotia, defined the known global distribution when the species was first recognized in 1922 (Bradford et al. 2004a; Bradford 2017). Neither the extent, nor the areas of occurrence of Atlantic Whitefish prior to settlement of Nova Scotia by Europeans is known (Bradford et al. 2004a, 2010). The present global distribution of the species is limited to three interconnected, semi-natural lakes (Milipsigate Lake, Minamkeak Lake, and Hebb Lake), which serve as the water supply for the town of Bridgewater in the Petite Rivière watershed, Nova Scotia (Edge 1987; Edge and Gilhen 2001; Fisheries and Oceans Canada 2018b). Atlantic Whitefish was listed as Endangered under SARA in 2003 and classified as “Critically Endangered” by the International Union for the Conservation of Nature, indicating that the species is at high risk for global extinction (Smith 2017).

Prior to the 2000s, little was known about the life history of Atlantic Whitefish. Lake-dwelling Atlantic Whitefish is unlike Lake Whitefish (*Coregonus clupeaformis*) in that it favours warmer surface waters (Edge and Gilhen 2001). The Tusket-Annis River population was historically anadromous, but the species has not been observed in that system since 1982 (Edge 1984; Bradford et al. 2004a). Less is known about the habitat requirements of juvenile Atlantic Whitefish as there have been few juveniles observed in the wild. Spawning locations, and therefore spawning habitat requirements, are unknown; however, small numbers of post-yolk sac larvae have been collected yearly since 2015 (Fisheries and Oceans Canada 2018c). Historical records indicate that Atlantic Whitefish were caught in coastal waters outside of the Tusket and Petite Rivière watershed, specifically in Yarmouth Harbour (June 1940), Hall’s Harbour (May 1958), and the mouth of the Sissiboo River (September 1919; Scott and Scott 1988). In 1995 and 1997, a single Atlantic Whitefish was caught in the Lahave River Estuary (COSEWIC 2010b).

Numerous threats to Atlantic Whitefish have been identified including non-native species, barriers to fish passage, and acidification from abandoned mines or quarries (Bradford et al. 2004b; Fisheries and Oceans Canada 2018b). Chain Pickerel (*Esox niger*) and Smallmouth Bass (*Micropterus dolomieu*) have been illegally introduced in the watershed where Atlantic Whitefish occurs, presenting a threat through competition and predation (COSEWIC 2010b; Fisheries and Oceans Canada 2018b). Fish passage was constructed at the Hebb Lake Dam in 2012 to improve connectivity and recreate conditions that allow for an anadromous life history (Fisheries and Oceans Canada 2016b); however, the other two lakes (Milipsigate and Minamkeak) remain physically isolated by dams. Unlike most other watersheds in Nova Scotia, paleolimnological work in the Upper Petite Rivière indicated that Atlantic Whitefish habitat has not previously been stressed by acid precipitation and that recent climate warming may be changing lake conditions (Ginn et al. 2008).

The current wild population size of Atlantic Whitefish is extremely small, with an estimated effective population size of 18–38 individuals (Cook 2012). Efforts have been directed towards preserving the remaining stocks of wild Atlantic Whitefish and developing a better understanding of the life history of this unique species, including captive-breeding efforts (Whitelaw et al. 2015; Fisheries and Oceans Canada 2016b). An important objective for achieving recovery is to increase the number and range of viable populations through the establishment of additional populations through translocation and possibly repatriation of an anadromous run in the Petite Rivière and (or) Tusket-Annis river systems (Fisheries and Oceans Canada 2006, 2016b; Bradford 2017). In 2000, Atlantic Whitefish were successfully bred in captivity for the first time at the Mersey Biodiversity Facility in Milton, Nova Scotia, using five wild adult individuals (Bradford et al. 2015; Whitelaw et al. 2015). Thereafter, collections of wild individuals

continued to maintain a captive population of approximately 30 adults (Bradford et al. 2015; Whitelaw et al. 2015).

Given the success of raising and breeding Atlantic Whitefish in captivity, a decision was made to introduce surplus first-generation fish to the Petite Rivière, below the Hebb Lake Dam, and Anderson Lake located in Dartmouth, Nova Scotia (Bradford et al. 2015). Over the span of 8 years (2005–2012), more than 12 000 captive-reared Atlantic Whitefish were introduced to Anderson Lake. Anderson Lake was not occupied historically by Atlantic Whitefish. This lake was chosen for a managed introduction of Atlantic Whitefish because it offered similar habitat characteristics (e.g., water chemistry, temperature) to the Petite Rivière, contained a potential prey source for Atlantic Whitefish (Rainbow Smelt *Osmerus mordax*), was devoid of threats posed by non-native Chain Pickerel and Smallmouth Bass, and was proximate to the Bedford Institute of Oceanography, which offered operational benefits (Bradford et al. 2015). The overall goal of the managed introduction was to evaluate the feasibility of using captive-reared fish to establish reproducing lake-resident populations of Atlantic Whitefish (Fisheries and Oceans Canada 2018b). Between 2008 and 2012, 42 introduced fish were observed that showed signs of maturation (Bradford et al. 2015); however, recent surveys in Anderson Lake and the Petite Rivière in 2016 and 2017 did not detect Atlantic Whitefish.

Along with providing a source of Atlantic Whitefish for reintroduction, the Mersey Biodiversity Facility also provided individuals for research purposes and has improved the understanding of Atlantic Whitefish biology (e.g., Hasselman et al. 2007, 2009; Cook 2012). For example, experiments with captive-raised Atlantic Whitefish were performed to understand the response of early life stages to differing levels of pH, salinity, and temperature, demonstrating that juvenile Atlantic Whitefish can tolerate pH levels down to 4.5, are fully tolerant of and, if given the choice, will selectively move into sea water and have an optimum growth temperature of 16.5 °C (Cook et al. 2010). In addition, a hydroacoustic tracking study was performed in Hebb and Anderson lakes on hatchery-raised and wild Atlantic Whitefish after intentional releases (Cook et al. 2014). The study indicated that hatchery-raised fish remained close to their point of introduction and tended to swim at the surface during daylight hours, making easy prey for visual predators like Osprey (*Pandion haliaetus*) and Common Loon (*Gavia immer*) (Cook et al. 2014). Although the captive-breeding program at Mersey Biodiversity Facility was successful, the facility was decommissioned and all remaining life stages, including broodstock (age 5+ and 6+), were released into Anderson Lake in 2012 (Bradford et al. 2015; Marshall et al. 2016).

Work is ongoing to identify sites for Atlantic Whitefish introductions (Bradford 2017; Fisheries and Oceans Canada 2018c). One previously suggested release site for Atlantic Whitefish is Oakland Lake, located northwest of Hebb Lake (Fisheries and Oceans Canada 2016b). Oakland Lake is a desirable candidate release site in that it is afforded protection from most major anthropogenic disturbances owing to its service as the public water supply, is currently free of predatory invasive species, and offers potential for anadromy via Oakland Stream which provides connectivity to the Mahone Bay estuary (Fisheries and Oceans Canada 2016b). Further studies are needed to confirm that environmental and biotic conditions of candidate release sites match those of the lakes known to support Atlantic Whitefish and, if desired, that sufficient connectivity with marine ecosystems is available to support the anadromous life-history component (Bradford 2017; Fisheries and Oceans Canada 2018c).

Channel Darter—Lake Erie, Lake Ontario, and St. Lawrence River DUs

Channel Darter is a small (average adult TL = 4.5 cm; Holm et al. 2009) cool/warm-water percid that occupies the benthic zone of

lakes and rivers (Scott and Crossman 1973; Coker et al. 2001). The distribution of Channel Darter in Canada is restricted to southern Ontario and Québec, but the species is also found discontinuously across the central United States (Fisheries and Oceans Canada 2013). Channel Darter was first listed under SARA as Threatened in 2003; the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) recently recognized and assessed three separate DUs for Channel Darter based on genetics and geographic disjunction—Lake Erie (Endangered), Lake Ontario (Endangered), and St. Lawrence River populations (“Special Concern”; COSEWIC 2016a). The original federal species recovery strategy for Channel Darter identified the need to determine if there are extirpated or new sites suitable for threat mitigation and habitat restoration for potential re-establishment (Fisheries and Oceans Canada 2013; Table 1). However, these objectives only relate to the Ontario DUs (i.e., Lake Erie and Lake Ontario), not the St. Lawrence River DU, based on the recent COSEWIC assessments.

Targeted sampling in Ontario has detected Channel Darter in locations where it was previously thought to be extirpated including Lake St. Clair and Port Burwell and Rondeau Bay in the eastern basin of Lake Erie (Fisheries and Oceans Canada 2013; COSEWIC 2016a; Fisheries and Oceans Canada, unpublished data). The abundance of Channel Darter across its range appears to be declining since its listing in 2003 (Phelps and Francis 2002; Burkett and Jude 2015; COSEWIC 2016a); however, quantitative estimates of abundance and distribution are generally lacking. Many threats have been identified for Channel Darter, including excess sedimentation and nutrients, pollution, alteration of beach and shoreline habitats, invasive species, alteration of flow regimes, and habitat fragmentation and loss (Reid et al. 2005; Reid and Mandrak 2008; Bouvier and Mandrak 2010a; Fisheries and Oceans Canada 2013; COSEWIC 2016a).

Several studies have investigated habitat associations for Channel Darter. Spawning appears to occur in late spring to early summer (water temperature of 14.5–26 °C; Comtois et al. 2004; Reid 2004; Fisheries and Oceans Canada 2016c) over coarse substrate with moderate water velocity for riverine populations (Reid 2004; COSEWIC 2016a). Males become territorial during spawning, inhabiting pebble and cobble areas (Boucher and Garceau 2010a), often with a large boulder present (Winn 1953). Less is known about the habitat requirements for juvenile Channel Darter, as there have been few juveniles observed in the wild, potentially indicating that habitat preferences change across life stages. Adults, in contrast, have been sampled more frequently. In streams or rivers, adult Channel Darter can be found in moderate flows (Boucher et al. 2009; Boucher and Garceau 2010a) where riffles transition into deep, sand-bottomed runs or pool habitat (Reid et al. 2005); alternatively, in lakes, Reid and Mandrak (2008) captured adults in beach habitat of Lake Erie consisting of coarse sand and fine gravel substrates. Lane et al. (1996) identified that Channel Darter had strong associations with gravel and sand substrates, and a moderate association with silt substrate. Channel Darter overwintering habitat is currently unknown but is not believed to be limiting for the species.

Science to support species recovery has been ongoing for Channel Darter since its listing in 2003 (Fisheries and Oceans Canada 2013). This includes being identified for protection under several ecosystem-based recovery strategies, including the Essex–Erie region (EERT 2008), Walpole Island (Bowles 2005), Outardes Est and Gatineau watersheds (COSEWIC 2016a), and the Multi-species Action Plan for Point Pelee National Park and Niagara National Historic Sites (Parks Canada Agency 2016a). As well, water-management strategies based on minimum flow rates have been designed to protect Channel Darter during breeding and egg incubation periods in the Trent River (Reid et al. 2016; Fisheries and Oceans Canada 2016c). Post-breeding, age-structured population matrix models have been developed to assess allowable harm, identify the minimum area for population viability, and develop

population-based recovery targets (Venturelli et al. 2010a). Based on minimum viable population estimates, 6800–31 000 adults are needed to sustain discrete Channel Darter populations in rivers when the probability of a catastrophic decline (50% decrease in population size) is 0.05–0.10 per generation, which translates to 0.9–125.2 ha of suitable habitat respectively (Venturelli et al. 2010a).

Copper Redhorse

Copper Redhorse is a cool/warm-water catostomid and the only freshwater fish species endemic to Québec (COSEWIC 2014; Fisheries and Oceans Canada 2012a). The species was first listed under SARA as Endangered in 2004. Copper Redhorse is one of few North American freshwater fish species that relies primarily on molluscs for prey (Mongeau et al. 1986, 1992). Compared to other *Moxostoma* species in the region, Copper Redhorse is the largest (average adult TL = 60–70 cm), takes the longest to reach sexual maturity (~10 years), and has the longest lifespan (>30 years; Mongeau et al. 1986; COSEWIC 2014). The single Copper Redhorse population is located in the St. Lawrence River basin and its main tributaries (Rivière Richelieu, Rivière L’Acadie (tributary of Rivière Richelieu), Rivière des Mille Îles, Rivière des Prairies, Rivière Saint-François, and Rivière Maskinongé; Fisheries and Oceans Canada 2012a; COSEWIC 2014). In 2000, the Copper Redhorse population was estimated at approximately 500 individuals (Vachon and Chagnon 2004). The species is now considered extirpated from Rivière Yamaska and the Rivière Noire system (COSEWIC 2014). However, population-level genetic diversity appears to be relatively high, likely a result of their long lifespan (Bernatchez 2004; Lippé et al. 2006). The Richelieu River plays a critical role in Copper Redhorse life history since it is the only river, to date, with known spawning grounds and nursery habitat (COSEWIC 2014).

Given that Copper Redhorse is found among the most densely populated region of Québec, this species is faced with many anthropogenic threats including impacts from urbanization and industrialization (e.g., degradation of habitat, introduced species, flow alteration) and agriculture (e.g., contamination of surface water by pesticides, habitat fragmentation and loss, bank erosion, and turbidity; Fisheries and Oceans Canada 2012a). A unique challenge for Copper Redhorse is that spawning occurs during periods of receding water levels following the late-spring and early-summer freshets, which coincides with peak pesticide use among local farms between late June and early July in the Rivière Richelieu (Gendron and Branchaud 1997; COSEWIC 2014). Several studies have demonstrated that exposure to pesticides, particularly endocrine-disrupting chemicals, can impact the maturation of Copper Redhorse (Gendron and Branchaud 1997; Maltais and Roy 2014), and may be a factor in the low levels of reproductive success over the last several decades (de Lafontaine et al. 2002). The expansion of the invasive Tench (*Tinca tinca*) throughout the Copper Redhorse range may also pose a significant threat to this species (Avlijaš et al. 2018).

Several recovery initiatives have been undertaken for Copper Redhorse to improve habitat conditions and support the long-term persistence of the species, including captive breeding and population supplementation efforts (Fisheries and Oceans Canada 2012a). In 2001, a multi-species fish ladder was constructed at the Saint-Ours dam to allow unrestricted passage through the Rivière Richelieu to protected spawning grounds in the Chambly rapids within the Pierre-Étienne-Fortin Wildlife Preserve (Fisheries and Oceans Canada 2012a; COSEWIC 2014). Restoration efforts to improve habitat and outreach programs to educate the public about important Copper Redhorse habitats are ongoing (Fisheries and Oceans Canada 2012a). In 2004, breeding efforts were initiated by the Ministère des Ressources Naturelles et de la Faune du Québec at a Parks Canada facility and larvae were reared in nine ponds at the Baldwin–Coaticook Provincial Fish Culture Station (Fisheries and

Oceans Canada 2012a). From 2004 to 2018, approximately 3.6 million Copper Redhorse larvae and 230 000 age-0 juveniles were released into the Rivière Richelieu (MFFP (Ministère des Forêts, de la Faune et des Parcs), unpublished data). The goal of these and future supplementation efforts is to replace the ageing wild spawning individuals before extinction while trying to preserve at least 90% of the initial Copper Redhorse genetic diversity over a period of 100 years (Bernatchez 2004; Fisheries and Oceans Canada 2012a; Table 1). To help achieve these objectives and to address problems encountered during the artificial breeding activities (e.g., high recapture rates, lack of males), the breeding, rearing, and stocking protocols were reviewed and refined (Vachon 2010), and semen cryopreservation techniques have been developed (Vachon 2018, Vachon et al. unpublished data).

Annual young-of-year (YOY) monitoring of redhorses in the Richelieu River combined with parentage genetics analysis are the main approaches, considered the least damaging, to assess persistence and recovery of the species (Fisheries and Oceans Canada 2012a). Survival of stocked juveniles up to age-2 has been confirmed with these methods (COSEWIC 2014), and subadults (TL < 500 mm) and young spawning individuals seem to be more prevalent since 2016 (MFFP, unpublished data). Continuing of the artificial breeding program, YOY monitoring efforts, and genetic analyses are needed to confirm whether stocked Copper Redhorse are reproducing in the wild and to re-evaluate the status of the entire species. If stocking efforts have been successful, but the goal of 4000 mature adults has not been reached, reintroduction efforts remain warranted for the Rivière Richelieu.

Eastern Sand Darter—Ontario DU

Eastern Sand Darter is a small, warm-water benthic percid (average adult TL = 6 cm; Coker et al. 2001; Holm et al. 2009) restricted to northeastern North America. Eastern Sand Darter was first listed under SARA as Threatened in Canada in 2003 (COSEWIC 2009a; Fisheries and Oceans Canada 2012b), and it is restricted to 17 geographically discontinuous populations within Ontario and Québec (COSEWIC 2009a). The Ontario and Québec populations were considered as separate DUs in 2009 (COSEWIC 2009a) and, as a result, both have their own recovery strategy (Fisheries and Oceans Canada 2012b; Fisheries and Oceans Canada 2014a). In Ontario, Eastern Sand Darter is extant in lakes Erie (including Rondeau Bay—observed in 2018 and 2019) and St. Clair; in the Sydenham, Grand, and Thames rivers; within Big Creek; and, in West Lake of the Lake Ontario drainage (Holm and Mandrak 1996; Dextrase et al. 2014a; Reid and Dextrase 2014). Eastern Sand Darter has been extirpated from Big Otter Creek, Catfish Creek, and Ausable River (Fisheries and Oceans Canada 2012b). In Québec, Eastern Sand Darter was collected from Lac St. Pierre, Rivière des Mille-Îles, Rivière L'Assomption, Rivière Ouareau, Lac Champlain, Rivière aux Saumons, Rivière Richelieu, and Rivière Trout between 2000 and 2010 (Fisheries and Oceans Canada 2014a). Recent sampling efforts revealed extant populations of Eastern Sand Darter in the Châteauguay, Yamaska, and Saint-François rivières, as well as in other historically occupied rivers (Yamachiche, Bécancour, Gentilly, aux Orignaux, and du Chêne rivières). New populations have been discovered in several rivers (Saint-Maurice, Champlain, Nicolet, Nicolet Sud-Ouest, Loup, Mascouche, Maskinongé, and Noire rivières; Alain Kemp, personal communication).

Eastern Sand Darter feeds primarily on larval insects (Turner 1921; Scott and Crossman 1973; Spreitzer 1979; Finch 2009) and is typically found in shallow areas of clear to tea-coloured waters of lakes and streams (<1.5 m; COSEWIC 2009a; Fisheries and Oceans Canada 2014a). The species occupies depositional zones and other areas with a high content of sand or fine-gravel substrates (Daniels 1993; Dextrase et al. 2014a). Research has demonstrated that YOY growth is significantly higher in sand-dominated reaches relative to silt-dominated habitats and that positive associations exist between

annual flow and YOY growth (Drake et al. 2008). The decline of Eastern Sand Darter populations has been attributed to threats related to the integrity of sand and fine-gravel habitats; for example, shoreline modification, turbidity and sediment loading, and altered flow regimes resulting from agricultural and urban development and impoundments (Boucher and Garceau 2010b; Bouvier and Mandrak 2010b; Dextrase et al. 2014a). Round Goby (*Neogobius melanostomus*) has invaded extant Eastern Sand Darter sites in Ontario and the abundance of Round Goby (Poos et al. 2010) was inversely correlated with abundance of Eastern Sand Darter in a tributary of Lake Erie (Raab et al. 2018). It has been hypothesized that Round Goby in the Great Lakes and their tributaries may be outcompeting Eastern Sand Darter for space and food resources (Bouvier and Mandrak 2010b); however, field or laboratory experiments to determine the mechanism of interaction have yet to be undertaken. In Québec, low water levels in the St. Lawrence River and the effect of waves from large ships can cause bank erosion, impacting the integrity of Eastern Sand Darter habitat (Boucher and Garceau 2010b).

Estimates of population size are not available for Canadian Eastern Sand Darter populations, and most local population trajectories are unknown due to the lack of standardized monitoring (Bouvier and Mandrak 2010b). Population modelling based on one of the largest known populations of Eastern Sand Darter in Ontario demonstrated that population growth rates were most sensitive to YOY survival (Finch et al. 2017). In addition, at least 4244–52 500 adult Eastern Sand Darter are needed to sustain a minimum viable population size given a probability of catastrophic event of 5% or 15%, respectively (Fisheries and Oceans Canada 2011). Furthermore, if eight discrete populations can be sustained at or above the minimum viable population size, the risk of extinction in Canada is estimated to be 5% (Fisheries and Oceans Canada 2011). Recent genetic analyses across the species range demonstrated that, contrary to previous views, Eastern Sand Darter shows stratified patterns of dispersal among sand bars at depositional river bends leading to low levels of population genetic differentiation within rivers; alternatively, large-scale population differentiation patterns exist across the species range resulting from the influence of historical, postglacial drainage patterns (Ginson et al. 2015), indicating a reduced likelihood of genetic rescue from geographically distant populations.

Science to support species recovery is ongoing for Eastern Sand Darter and its habitat and includes two multi-species, ecosystem-based action plans in Ontario (Fisheries and Oceans Canada 2018d, 2018e). These plans aim to prioritize management actions to support the recovery of imperilled freshwater species in the region, including Eastern Sand Darter. Although both DUs are listed as Threatened, only the recovery strategy for the Ontario population identifies supplementation or repatriation as a potential approach to achieve recovery (Fisheries and Oceans Canada 2012b, 2014a; Table 1). Efforts to reintroduce Eastern Sand Darter have not been attempted in Ontario; however, recent field and modelling studies have been conducted to refine knowledge of habitat associations and identify suitable repatriation sites in locations where the species has been extirpated. Results suggest that historically occupied tributaries of the Great Lakes could sustain Eastern Sand Darter populations in Ontario (Dextrase et al. 2014a; Lamothe et al. 2019); however, natural recolonization is unlikely due to dispersal limitations (COSEWIC 2009a), including the lack of local source populations proximate to historical sites. Based on site-, reach-, and watershed-level habitat characteristics (Dextrase et al. 2014a) and species co-occurrence patterns (Lamothe et al. 2019), sites that historically contained Eastern Sand Darter within sections of the Ausable River and Big Otter Creek could serve as potential reintroduction sites in Ontario, and the Grand River and (or) Thames River populations may be the only rivers with sufficiently large, self-sustaining populations to allow removals for the

purposes of captive-breeding, translocation, or reintroduction efforts.

Gravel Chub

Gravel Chub is a small-bodied (average adult TL = 7.5 cm; Holm et al. 2009), cool-water minnow last captured in Canada in 1958 (Coker et al. 2001; Fisheries and Oceans Canada 2016d). It was listed as Extirpated under SARA in 2003. Very little is known about the biology of Gravel Chub, particularly in Canada (Edwards et al. 2007). This species is believed to feed on phytoplankton, macrophytes, molluscs, and benthic insects, and reproduces over gravel substrates in riffles, with no parental care (Coker et al. 2001). Prior to extirpation, Gravel Chub was present in the Thames River near Muncey and further downstream in southwest Middlesex County (formerly Mosa Township) near Moraviantown, Ontario (Holm and Crossman 1986; Edwards et al. 2007; Fisheries and Oceans Canada 2016d). The cause of Gravel Chub extirpation in Canada is unknown; however, extirpations in other parts of the global range have been associated with siltation (e.g., Ohio; Trautman 1957), an ongoing stressor in the Thames River drainage (LTVCA 2018).

Historically, the upper Thames River was clear, with fast flows and sand to gravel substrates (Parker and McKee 1987; Parker et al. 1988a), which coincides with preferred Gravel Chub habitat elsewhere in North America (Parker and McKee 1987). However, the Thames River continues to exhibit widespread habitat degradation, including high levels of phosphorus and *Escherichia coli*, despite substantial efforts to restore the historically degraded watershed (LTVCA 2013, 2018). Sedimentation remains a significant problem as a result of widespread and intensive agricultural activity, although ongoing reforestation efforts may be helping to counteract expanding urbanization and agricultural pressures. One short-term objective identified in the Gravel Chub recovery strategy is to examine the feasibility of relocations, captive rearing, and re-introductions (Table 1) (Fisheries and Oceans Canada 2016d). However, habitat improvements need to occur in the region before undertaking reintroduction efforts for Gravel Chub, particularly related to sediment loads and turbidity. Furthermore, research is needed to better understand life-stage requirements for Gravel Chub, particularly for YOY and juvenile life stages to evaluate whether a potential habitat bottleneck exists for the species. As well, research is needed to better understand Gravel Chub reproductive biology if efforts proceed to breed Gravel Chub in captivity. If improvements in habitat conditions are made, consideration would be needed to determine if translocations from nearby US populations are feasible, the implications of such efforts (e.g., genetic), or if captive-breeding efforts need to be initiated.

Lake Chubsucker

Lake Chubsucker is a moderately sized (average adult TL = 20 cm; Holm et al. 2009), warm-water catostomid and the only member of the genus *Erimyzon* in Canada (Mandrak and Crossman 1996; COSEWIC 2008). It was first listed as Threatened under SARA in 2003 then as Endangered in 2011. Lake Chubsucker is omnivorous, preferring to feed on benthic crustaceans, insects, algae, and plant material (Holm et al. 2009; Staton et al. 2010), and it primarily inhabits clear, still, and heavily vegetated waters such as floodplain lakes, marshes, and wetlands (Mandrak and Crossman 1996; COSEWIC 2008; Staton et al. 2010). Lake Chubsucker spawns in the spring between April and June when mature adult females (age 3+ years) lay between 3000 and 20 000 eggs on aquatic vegetation (Becker 1983; COSEWIC 2008). The distribution of Lake Chubsucker is discontinuous across North America with populations spanning the lower coastal plains from Texas to Virginia, northwards to the southern Great Lakes basin (Staton et al. 2010). In Canada, Lake Chubsucker is restricted to southern Ontario in the

Old Ausable Channel and L Lake in the Lake Huron basin, Lake St. Clair and Lake Erie coastal wetlands and dyked marshes, and the upper Niagara River watershed (Fisheries and Oceans Canada 2017a). The most robust population in Canada is believed to occupy L Lake (Fisheries and Oceans Canada 2018d).

Given the rapid losses of freshwater wetlands across North America (Gibbs 2000), including in the Great Lakes basin (Detenbeck et al. 1999), Lake Chubsucker is primarily threatened by the effects of wetland habitat degradation and habitat loss (COSEWIC 2008), including natural succession. Extirpations of Lake Chubsucker in its Canadian range have likely occurred in Jeanette's Creek in the Thames River watershed, lower Ausable River, Big Creek (Norfolk Co.) watershed, and a tributary of the Niagara River (Tea Creek; Staton et al. 2010). The cause of these extirpations has been attributed to the effects of siltation and increased turbidity associated with the channelizing of the Ausable River and agricultural practices in southwestern Ontario (COSEWIC 2008), which has reduced the availability of submerged macrophytes that the species relies on for reproduction, feeding, and cover. Many of the Big Creek tributaries where Lake Chubsucker was historically found have been channelized and converted into agricultural drains or completely buried (e.g., Silverthorn Creek; COSEWIC 2008). Moreover, invasive species, such as the Common Carp (*Cyprinus carpio*) and European Common Reed (*Phragmites australis*), present an ongoing threat to the integrity of Lake Chubsucker habitat in Ontario (COSEWIC 2008).

Efforts to recover Lake Chubsucker in southern Ontario are underway. Lake Chubsucker is currently supported under a federal recovery strategy and two federal multi-species action plans (Parks Canada Agency 2016a, 2016b; Fisheries and Oceans Canada 2018d), which provide detailed recovery planning that supports species recovery. Population models have been developed to identify strategic endpoints for management. For example, population matrix models suggest that recovery of Lake Chubsucker is dependent on reducing harm to early life stages of Lake Chubsucker (Vélez-Espino et al. 2009; Young and Koops 2011). Given a probability of catastrophic decline of 0.15 per generation, a reasonable estimate for Lake Chubsucker (Danylchuk and Tonn 2003; Reed et al. 2003), a minimum viable population size would need to consist of at least 2730 adults (Young and Koops 2011). Population sizes are currently unknown for Ontario populations of Lake Chubsucker.

Efforts to repatriate Lake Chubsucker have not been initiated in Ontario but are identified as a potential recovery measure in relevant recovery strategies (EERT 2008; Staton et al. 2010), specifically for populations in the Ausable River (Fisheries and Oceans Canada 2018d). Several uncertainties may delay the implementation of reintroduction efforts, including knowledge of habitat associations among all life stages, extent of genetic diversity among local populations (project ongoing), and the potential impact of removals (to support translocations) on source populations (Staton et al. 2010; Fisheries and Oceans Canada 2017a). Furthermore, detailed information on population size, structure, and condition is needed to better understand the robustness of source populations for potential translocations, particularly for the populations inhabiting L Lake and the Old Ausable Channel. Preliminary efforts are underway to develop husbandry practices and captive-breeding techniques of Lake Chubsucker at the University of Windsor, although formal reintroduction activities have yet to be planned.

Northern Madtom

Northern Madtom, a small (average adult TL = 8 cm; Holm et al. 2009), warm-water member of the Ictaluridae family, is a globally rare species and one of the rarest species in Canada (Edwards et al. 2012). It was first listed as Endangered under SARA in 2003 (Edwards et al. 2012). As a nocturnal opportunistic feeder, the

species seeks refuge in benthic cover during the day and forages on benthic invertebrates and small prey fishes by night (Holm and Mandrak 1998; Holm et al. 2009). Based on limited observations of Northern Madtom in the wild in Canada, the species appears to be found in small to large rivers and lakes, in clear to turbid waters, with moderate to fast currents, and across a variety of substrates (Dextrase et al. 2003; Manny et al. 2014). However, given its restricted distribution, there may be microhabitat characteristics critical to its survival that remain undescribed (Dextrase et al. 2003; COSEWIC 2012a).

Spawning of Northern Madtom occurs in mid- to late-July (Taylor 1969; MacInnis 1998) and, similar to other catfishes, the species spawns in cavities and eggs are guarded by the male (Burr and Mayden 1982; MacInnis 1998). In Canada, Northern Madtom is at the northernmost extent of its distribution (Goodchild 1993) with extant populations in the Detroit, St. Clair, and Thames rivers and Lake St. Clair, and is believed to be extirpated in the Sydenham River (Holm and Mandrak 1998; COSEWIC 2012a; Edwards et al. 2012). Environmental DNA sampling in the Sydenham River in 2013 identified seven sites containing Northern Madtom DNA (Balasingham et al. 2018); field sampling using conventional gear is ongoing to confirm the presence of Northern Madtom individuals. Sampling in the Detroit River using baited minnow traps found that Northern Madtom was the most abundant species in the minnow traps after the invasive Round Goby, where catch per unit effort of Northern Madtom varied between 0.07 and 0.58 individuals per minnow-trap day across sites (Manny et al. 2014).

Detailed life-history information for Northern Madtom is generally lacking across the species' range and what is known tends to be from limited observations of Northern Madtom in the wild. Previously, Northern Madtom was thought to be a short-lived species, with maximum reported ages of approximately 2–3 years (Taylor 1969; COSEWIC 2012a), but recent captures from the Detroit River found individuals of at least 6 years of age (Manny et al. 2014). Given the prevalence of Northern Madtom in the Detroit River, it may be tolerant to some types of pollution (Goodchild 1993; Holm and Mandrak 1998), although no empirical research exists to substantiate this assumption. Threats to Northern Madtom persistence in the wild are believed to include siltation, turbidity, nutrients, and invasive species (e.g., Round Goby), but no empirical studies have examined these effects (COSEWIC 2012a; Edwards et al. 2012). A primary challenge for studying Northern Madtom in the wild, not unlike most SARA-listed fishes, is the combination of species rarity and the need for novel, active sampling approaches (e.g., trawling) that can be more easily used to identify unique species-specific habitat associations relevant for habitat protection and reintroductions.

One objective from the Northern Madtom recovery strategy is to determine the feasibility of relocations and captive rearing (Edwards et al. 2012; Table 1). The only likely viable source populations for translocations would be from the upper Detroit River or St. Clair River (Dextrase et al. 2003; Manny et al. 2014), but research is needed to understand the genetic and population implications (e.g., effects of removal) of such an effort. Northern Madtom would benefit from increased directed monitoring efforts to provide a better understanding of its distribution, habitat requirements, genetic structure, and movement patterns. Currently, there are no known efforts to breed Northern Madtom in captivity. However, Smoky Madtom (*Noturus baileyi*) and Yellowfin Madtom (*Noturus flavipinnis*) captive-breeding programs have resulted in successful reintroductions in the southeastern United States (Shute et al. 2005).

Pugnose Shiner

Pugnose Shiner, a small (average adult TL = 5 cm; Holm et al. 2009), globally rare, warm-water minnow, is found discontinu-

ously through northeastern North America including in southern Ontario (Parker et al. 1987; Coker et al. 2001; COSEWIC 2013) and has been listed as Endangered under SARA since 2005. Pugnose Shiner is distributed in five distinct regions of southern Ontario, including the Lake Huron basin (Old Ausable Channel, Teeswater River), Lake St. Clair basin (Lake St. Clair, St. Clair National Wildlife Area), Lake Erie basin (Long Point Bay), Lake Ontario basin (East Lake, South Bay, Trent River, Waupoos Bay, West Lake, Wellers Bay), and the St. Lawrence River (Bouvier et al. 2010). Extirpations of Pugnose Shiner have likely occurred in Point Pelee National Park and Rondeau Bay in the Lake Erie basin, and possibly the Gananoque River, a tributary to the St. Lawrence River (Bouvier et al. 2010).

Pugnose Shiner is primarily found in clear, slow-moving rivers and lakes where there is an abundance of rooted aquatic vegetation (Scott and Crossman 1973; Leslie and Timmins 2002; COSEWIC 2013). Aquatic vegetation is a key component of Pugnose Shiner habitat (McCusker et al. 2014a), as the species broadcast spawns over submerged plants that provide shade for the highly photophobic eggs (Leslie and Timmins 2002). Submerged aquatic vegetation also provides important cover from predators for all life stages. The diet of Pugnose Shiner consists of decomposing organic matter (Goldstein and Simon 1999) and small cladocerans, leeches, and caddisfly larvae (Holm and Mandrak 2002).

The greatest threats to Pugnose Shiner populations in Canada are the loss and degradation of preferred habitat due to aquatic vegetation removal, nutrient and sediment loading, and increased turbidity (Bouvier et al. 2010). Several studies suggest that the habitat of Pugnose Shiner has been depleted as a result of increased sediment loading and turbidity (Bailey 1959; Fisheries and Oceans Canada 2012c). Common Carp also present a risk to Pugnose Shiner habitat viability, as this species disturbs the sediment when feeding, resulting in increased turbidity and uprooted aquatic vegetation (Fisheries and Oceans Canada 2012c). Gray et al. (2014, 2016) experimentally showed that turbidity had a greater negative effect on the behaviour and physiology of Pugnose Shiner than less imperilled, closely related species. Furthermore, as water levels decrease with climate change in Lake Erie, the invasive European Common Reed is expected to expand beyond its current range, potentially leading to the total loss of Pugnose Shiner habitat in Long Point Bay (McCusker 2017; Megan R. McCusker, unpublished data).

A federal recovery strategy has been developed for Pugnose Shiner (Fisheries and Oceans Canada 2012c) and the species is included in two federal multi-species action plans (Parks Canada Agency 2016a, 2016b; Fisheries and Oceans Canada 2018d). These plans describe current and future efforts to protect habitat, including important wetland habitats for Pugnose Shiner, and involve local stewardship activities to educate and promote efforts to recover species at risk in southern Ontario. Reintroduction efforts have not been initiated for Pugnose Shiner, but they are identified as a potential recovery action (Fisheries and Oceans Canada 2012c; Table 1). Population genetic analyses of samples from throughout the range confirmed low levels of connectivity between populations and that genetic rescue is unlikely for Ontario populations (McCusker et al. 2014b). Population modelling for Pugnose Shiner in Ontario estimated a minimum viable population size of 1929–14 325 adults when the probability of a catastrophic decline was 0.05–0.10 per generation, respectively (Venturelli et al. 2010b). Minimum viable area for Pugnose Shiner population viability is estimated to be between 2000 m² and 15 000 m² of river habitat or between 7000 m² and 50 000 m² of lake habitat (Venturelli et al. 2010b). Given the risks to early life stage Pugnose Shiner (Venturelli et al. 2010b), recovery efforts should focus on protecting early life stage habitat, particularly areas of aquatic vegetation. Further, re-establishing populations at the three extirpated sites could reduce the risk of extinction to 3.5% over the next 250 years; without these three populations, the

risk of extinction increases to approximately 9.5% (Venturelli et al. 2010b).

The cause of likely extirpations of Pugnose Shiner in Ontario vary. Changing trophic dynamics in Point Pelee National Park and Rondeau Bay resulting from newly established species may have played a role (e.g., Black Crappie *Pomoxis nigromaculatus*, Bluegill *Lepomis macrochirus*, and Brook Silverside *Labidesthes sicculus*; Holm and Mandrak 2002; Surette 2006) along with issues of nutrient loading (EERT 2008). Further sampling is needed to better understand the current fish community dynamics in Point Pelee National Park prior to planning reintroduction efforts at this location. Bouvier et al. (2010) identified the consequences of habitat modifications, including the effects of aquatic-vegetation removal, nutrient loading, and sedimentation to Pugnose Shiner as high in Rondeau Bay, indicating that large-scale habitat restoration is needed prior to reintroduction efforts. Finally, little is known about the suitability of the Gananoque River for reintroduction (Bouvier et al. 2010), indicating the need for further assessment of that location from a reintroduction perspective.

Given the geographically and genetically disjunct nature of Pugnose Shiner populations (McCusker et al. 2014b), reintroduction efforts would likely have to focus on translocations from donor populations within Ontario or to develop captive-breeding programs for the species. Research to identify donor populations has yet to be initiated. The Pugnose Shiner population in the St. Lawrence River has the largest effective population size and genetic diversity (McCusker et al. 2014b, 2017) and, therefore, may be the most robust donor population. However, modelling efforts are needed to understand how translocations could impact both source and recipient populations. Active captive-breeding efforts for Pugnose Shiner are ongoing at the Freshwater Restoration Ecology Centre at the University of Windsor. As well, several researchers at McGill University are conducting laboratory research on wild-stock Pugnose Shiner collected from ponds at SUNY Cobleskill, which were originally stocked with fish from Sodus Bay, Lake Ontario (Carlson et al., submitted for publication). The Cobleskill fish have also been used to successfully re-establish Pugnose Shiner in Chaumont Bay, Lake Ontario (Carlson et al., submitted for publication). Finally, a Pugnose Shiner population has been established in a stormwater retention pond in Illinois, stocked with fish from two lakes in the Fox River drainage, Illinois (Schaeffer et al. 2012).

Redside Dace

Redside Dace is a small (average adult TL = 7.5 cm; Holm et al. 2009), insectivorous, cool-water minnow endemic to North American streams. The species is discontinuously distributed across eastern North America (Parker et al. 1988b), with populations in Canada limited to 17 watersheds within the Lake Erie, Huron, and Ontario basins (Poos et al. 2012; Fisheries and Oceans Canada, 2019). The species was listed as Endangered under SARA in 2017 due to significant declines in population size over the last several decades (Parker et al. 1988b; Coker et al. 2001; Hutchings and Festa-Bianchet 2009; Poos et al. 2012; COSEWIC 2007, 2017). Redside Dace habitat consists of clear pools and slow-flowing riffles found in reaches of relatively small, meandering streams (Parker et al. 1988b; Andersen 2002), where it is often found near undercut banks in areas with abundant riparian grasses (McKee and Parker 1982; Andersen 2002). Redside Dace spawns in shallow gravel riffles as nest associates with species such as the Common Shiner (*Luxilus cornutus*) and Creek Chub (*Semotilus atromaculatus*), but is typically found in pools across a variety of substrate sizes from silt and detritus to boulders (Koster 1939; McKee and Parker 1982; Andersen 2002; Fisheries and Oceans Canada 2019).

Threats to Redside Dace are largely associated with habitat alteration and degradation related to urbanization and agricultural development (Parker et al. 1988b; Poos et al. 2012; COSEWIC 2017;

Reid and Parna 2017; Fisheries and Oceans Canada 2019), which includes the destruction of riparian habitat and subsequent problems with erosion control leading to greater rates of siltation and higher turbidity (McKee and Parker 1982; Parker et al. 1988b), changes to river channel morphology associated with increased peak flows in hardened watersheds (COSEWIC 2007), and other hydrological effects (e.g., reduced base flow; Drake and Poesch 2018). Significant alteration to flow and thermal regimes as a result of urban effects, such as stormwater modification, is also suspected in the species' decline. Redside Dace is a visual predator that feeds on terrestrial insects (Koster 1939; McKee and Parker 1982); the removal of riparian vegetation impacts water clarity and prey capture efficiency and the production of terrestrial insects (Reid et al. 2008). Other threats to Redside Dace populations in Canada include agricultural activities, baitfish harvesting, climate change, commercial water extraction, and non-native species introductions (Fisheries and Oceans Canada 2019). Extirpations of Redside Dace in southern Ontario have likely occurred in several watersheds where the effects of urbanization are widespread and considered irreversible including: Don River, Etobicoke Creek, Highland Creek, Mimico Creek, Morrison Creek, Petticoat Creek, Pringle Creek, Sheridan Creek, and a watershed on the Niagara peninsula (COSEWIC 2007, 2017; Fisheries and Oceans Canada 2019).

Efforts are underway to protect and restore Redside Dace populations. This includes efforts to identify candidate sites for species reintroduction. Given there are few streams where Redside Dace populations are considered relatively robust (Sixteen Mile Creek, East Humber River, some Rouge River tributaries; Fisheries and Oceans Canada 2019), these sites likely represent the only donor populations for translocations within its Canadian distribution. Assuming that Redside Dace individuals mature by age 2, population modelling suggested that the minimum population size of 2952 individuals is needed to maintain a 95% probability of persistence within the next 100 years, and 4295 individuals if Redside Dace mature at age 3 (Vélez-Espino and Koops 2008). Poos et al. (2012) extrapolated density-based sampling to determine that Redside Dace population abundance was 462–741 individuals in Gully Creek, 21 530–38 582 in the Humber River, 4499–9180 in the Rouge River, 1207–2398 in Duffins Creek, and 402–1607 in the Don River, indicating the potential for future population collapse in Duffins Creek, Gully Creek, and that already reported for the Don River (COSEWIC 2017). An additional 7.2, 10.7, and 7.6 km of optimal habitat has been suggested as a restoration target in the Don River, Duffins Creek, and Gully Creek, respectively, if Redside Dace is to maintain long-term viability at these sites (Poos et al. 2012).

Successful reintroduction of Redside Dace is complicated by its fragmented distribution. In most Ontario tributaries, Redside Dace is now restricted to headwater sections, often with poor connectivity within and among drainages. Genetic analysis found three distinct haplogroups among Redside Dace populations across the species' range (including within the United States), with very little to no gene flow occurring between local populations (i.e., tributary to tributary; Serrao et al. 2018). Most sites where Redside Dace have been extirpated have very poor habitat suitability, despite best management practices (Yates et al. 2007), largely due to continuing urban development (Reid and Parna 2017). Furthermore, given the continuing urban expansion within the Greater Toronto Area, it is unlikely that regions where Redside Dace have been extirpated will recover to conditions suitable for reintroduction efforts.

Efforts to reintroduce Redside Dace would benefit from a better understanding of juvenile habitat associations and seasonal changes in habitat (e.g., wintering habitat), as both could present roadblocks for reintroduction success (Vélez-Espino and Koops 2008; Poos and Jackson 2012). Formal captive-breeding programs do not currently exist for Redside Dace in Canada; however, the

species has been successfully bred in captivity and research is being performed to understand the population genetics (Pitcher et al. 2009a) and reproductive biology of the species (Pitcher et al. 2009b; Beausoleil et al. 2012). Furthermore, sperm cryopreservation techniques have been developed to preserve genetic diversity in certain populations (Butts et al. 2013).

Spring Cisco

Spring Cisco is a moderate-sized (average adult TL = 15–30 cm; Fisheries and Oceans Canada 2014b), cold-water ecomorphotype of the Cisco (*Coregonus artedii*; Coker et al. 2001; Turgeon and Bernatchez 2003) found only in Lac des Écorces of southwestern Québec. Spring Cisco was first listed as Endangered under SARA in 2013. North American ciscoes have a complex evolutionary history characterized by periods of geological and genetic convergence and divergence that blurs the genetic ancestry of the species, resulting in distinct ecomorphotypes across the species' ranges (Turgeon and Bernatchez 2003). Unlike other ciscoes, Spring Cisco spawn in the spring (Pariseau et al. 1983; COSEWIC 2009b) and display unique gill-raker morphology (average 43 gill-rakers; Hénault and Fortin 1989). There have been few studies on Spring Cisco given its geographic isolation, which limits our knowledge on the overall population trends; however, the relative abundance of Spring Cisco has been declining since the 1990s, and the average length of fish caught has decreased over that period (COSEWIC 2009b; Fisheries and Oceans Canada 2010b).

Lac des Écorces is comprised of two basins, with maximum depths of 23 m and 38 m, and it has an area of 6.58 km² (Fisheries and Oceans Canada 2010b, 2014b). This lake is unique in that it has a high turnover rate (seven times per year) mimicking riverine conditions (Pariseau et al. 1983; Fisheries and Oceans Canada 2014b), with temperatures much higher than the thermal optimum of most ciscoes (COSEWIC 2009b). Spring Cisco gather in the deep pools of Lac des Écorces in the spring to spawn, when temperatures are fairly low (<6 °C; temperatures >10 °C can be lethal to eggs; COSEWIC 2009b). As waters cool in the fall, Spring Cisco can be found in waters >12 m deep (Fisheries and Oceans Canada 2014b).

Lac des Écorces has been stocked with several game species including Brook Trout (*Salvelinus fontinalis*) and Walleye (*Sander vitreus*), and it has had unauthorized introductions of Largemouth Bass (*Micropterus salmoides*) and Rainbow Smelt (Fisheries and Oceans Canada 2010b). These introductions present several challenges to the protection and recovery of Spring Cisco via presumed predatory and competitive effects; predation by Rainbow Smelt on Spring Cisco larvae appears to be severely impacting the population (Fisheries and Oceans Canada 2010b). Several studies have demonstrated negative impacts of Rainbow Smelt invasions on native fish communities (Evans and Loftus 1987), including ciscoes (Selgeby et al. 1978; Loftus and Hulsman 1986; Hrabik et al. 1998). Residential development and agriculture around Lac des Écorces is also deteriorating habitat quality of Spring Cisco (Fisheries and Oceans Canada 2010b, 2014b). Particularly relevant threats to Spring Cisco include increased nutrient loading from local agriculture and discharge of wastewater, and sedimentation from shoreline degradation; as of 2010, approximately 53% of shoreline areas were completely vegetated and 11% of the properties around the lake showed high risk of pollution from private sewage treatment systems (Séguin 2010; Fisheries and Oceans Canada 2014b).

Several projects to support the recovery of Spring Cisco are underway. Between 2010 and 2016, removals of spawning Rainbow Smelt in Lac des Écorces tributaries resulted in over 7500 kg of smelt removed from the system (Fisheries and Oceans Canada 2014b); however, after 7 years, it was concluded that removal was not effective to reduce the Rainbow Smelt population (Louise Nadon, unpublished data). As well, shoreline protection strategies

and wastewater regulations have been implemented in the Lac des Écorces region and should lead to improved water quality (Fisheries and Oceans Canada 2014b).

Supplementation of Spring Cisco populations represents a high priority potential recovery action that has yet to be initiated (Table 1; Fisheries and Oceans Canada 2014b). As well, the development of a “sanctuary” or “ark population” (i.e., introducing Spring Cisco into another body of water) has been recommended as a potential recovery action; however, captive breeding has not been initiated and a suitable waterbody for Spring Cisco introductions has yet to be identified. Captive breeding of Spring Cisco is likely feasible given the success of captive-breeding efforts for other cisco species (e.g., Bloater—*Coregonus hoyi*; Presello et al. In Press); however, a suitable waterbody to introduce Spring Cisco is needed that includes access to physical and thermal habitat where spawning can occur and a suitable fish community without invasive species prior to reintroduction (Fisheries and Oceans Canada 2014b). Initial searches for introduction sites should start within the southwestern Québec region given its historical distribution and difficulty in transporting fish long distances.

Striped Bass—St. Lawrence River DU

Striped Bass is a moderately sized (average adult TL = 35–50 cm) temperate bass distributed along the Atlantic coast of North America, from northeast Florida to the St. Lawrence River (COSEWIC 2012b). Four DUs of Striped Bass are recognized in Canada including the southern Gulf of St. Lawrence DU, Bay of Fundy DU, St. Lawrence Estuary DU, and St. Lawrence River DU (COSEWIC 2004, 2012b). In November 2012, COSEWIC assessed the southern Gulf of St. Lawrence DU as a species of Special Concern, the Bay of Fundy DU as Endangered (COSEWIC 2012b), and the St. Lawrence River DU as Endangered, but none are currently protected under SARA. In contrast, the St. Lawrence Estuary DU, assessed only in 2004 (COSEWIC 2004), is listed as Extirpated under SARA (Fisheries and Oceans Canada 2017b) (Table 1), however, this DU would more appropriately be listed as Extinct, as it is found nowhere else in the world.

Striped Bass is an anadromous species, with most of its adult life spent in coastal, estuarine, and saltwater environments. Adults typically enter estuaries or freshwater habitats in the fall to spend the winter (COSEWIC 2012b), but they have also been observed wintering in high-flow marine areas (Keyser et al. 2016). In Canada, Striped Bass spawn between late May and early June in rivers (Miramichi River, Shubenacadie River, Rivière du Sud and the baie de Beauport area; Fisheries and Oceans Canada 2017c). In the St. Lawrence River, soon after hatching, YOY are found near the spawning grounds and in the oligohaline waters of the estuarine turbidity maximum zone. In the late summer, Striped Bass tend to disperse further downstream in search of new foraging opportunities (Vanalderweireldt et al., 2019). Subadults (between YOY and adult) are usually found in brackish waters of estuaries (Secor and Piccoli 1996; Martino 2008). Striped Bass undergo metaphoetesis, starting with a diet of zooplankton as smaller juveniles and becoming more opportunistic as adults, including piscivory (Robitaille et al. 2011). Larval survival has been directly related to available zooplankton abundance (Martin et al. 1985). Throughout its range, Striped Bass is well-known as an important commercial (e.g., Koo 1970; Richards and Rago 1999) and recreational sport fish (Lothrop et al. 2014), including in Canada (Andrews et al. 2017; MFFP 2018).

The original St. Lawrence Estuary DU was extirpated in the mid-1960s due to overexploitation by fishing and habitat degradation and loss (Beaulieu et al. 1990; Pelletier et al. 2011). Captive-breeding efforts at the Baldwin-Coaticook hatchery in Québec with broodstock of Southern Gulf origin (Miramichi River) and subsequent reintroduction efforts were initiated in 2002 (efforts began before the initiation of SARA) and have led to the re-

establishment of Striped Bass in the St. Lawrence River (Fisheries and Oceans Canada 2017c); however, they are not considered part of the original St. Lawrence Estuary DU (COSEWIC 2012b). The Southern Gulf population was chosen due to its proximate location and northern extent (Pelletier et al. 2011). Between 2002 and 2018, 3001 spawning individuals, 18 268 juveniles, and 34.5 million larval Striped Bass were released into the St. Lawrence River (MFFP, unpublished data). Among those fish, many were implanted with a tag to identify year class, release location, and release date, or were marked with oxytetracycline (Pelletier et al. 2011). Confirmation of Striped Bass spawning in the St. Lawrence River occurred in 2008 (Bourget et al. 2008; Pelletier et al. 2011). Spawning locations include areas in the Rivière-du-Sud basin and the Québec port area for fishes age-3 to age-10 (Bujold and Legault 2012; COSEWIC 2012b; Valiquette et al. 2017). As the reintroduced population now shows signs of recovery, it was decided to stop stocking activities for 2019 and 2020; however, Striped Bass stocks will be maintained, as a precaution, at the Baldwin-Coaticook hatchery to retain the acquired expertise on captive breeding and maintaining fish stocking capacity.

Successful spawning in the St. Lawrence River only 6 years after reintroduction indicates early success of the reintroduction program; however, the long-term trajectory of this population depends on several uncertainties. Striped Bass is known to have irregular recruitment and is dependent upon favourable environmental conditions and prey availability to ensure survival of early life stages (Kimmerer et al. 2000; Martino and Houde 2012). Specifically, egg survival is closely linked to temperature, dissolved oxygen, and the presence of moderate current at incubation sites (Robitaille et al. 2011). Present threats to the St. Lawrence population include threats to habitat (e.g., dredging), harvesting (e.g., bycatch or targeted mortality), and biological threats (e.g., parasites; Fisheries and Oceans Canada 2017b, 2017c).

Studies are currently underway to better understand the ecology and life history of the new St. Lawrence River population. Telemetry has demonstrated that, unlike the historical population of Striped Bass thought to winter in Lac St. Pierre (Robitaille 2010), the introduced population winters in the middle estuary, south of Isle-aux-Grues and near Québec City (Valiquette et al. 2017). As well, Striped Bass have been observed wintering around Lac St. Pierre and telemetry receivers have been deployed in this area to provide more information about their winter behaviour (Éliane Valiquette, 2018, personal communication). However, more research is needed to better understand the dynamics of the newly formed breeding population in the St. Lawrence River, including fecundity, survival of different life stages, and the extent of the juvenile and adult distribution (Fisheries and Oceans Canada 2017c), to determine if, or how, future reintroductions of this species should proceed.

Westslope Cutthroat Trout—Alberta DU

Cutthroat Trout (*Oncorhynchus clarkii*) is a polytypic species native to western North America consisting of more than a dozen described subspecies (Allendorf and Leary 1988), including the Westslope Cutthroat Trout (*O. c. lewisi*). Westslope Cutthroat Trout inhabits cool, clean, and well-oxygenated waters, with high phenotypic variation in size (average adult TL = 20–40 cm), colour, and life-history strategies (migratory versus non-migratory) across its range (Fisheries and Oceans Canada 2014c, 2017d; COSEWIC 2016b). Westslope Cutthroat Trout feeds primarily on aquatic invertebrates including Ephemeroptera, Plecoptera, and Trichoptera (Schoby and Keeley 2011), which are indicators of stream habitat quality (Barbour et al. 1992). There are two DUs of Westslope Cutthroat Trout in Canada: the Alberta DU (Threatened) and the Pacific DU (Special Concern) in British Columbia (COSEWIC 2016b). The COSEWIC assessment and subsequent listing of the species under SARA includes only native, genetically pure (≥99% pure)

Westslope Cutthroat Trout within its historical natural distribution. Both Westslope Cutthroat Trout DUs in Canada are at the northern extent of the subspecies range and are adapted to cold waters with low productivity (Rasmussen et al. 2012; Yau and Taylor 2013, 2014). Recent genetic research indicates that the two Canadian DUs originated from the same lineage (neoboreal clade; Young et al. submitted for publication).

The Alberta Westslope Cutthroat Trout DU was historically abundant, occupying approximately 274 streams within the Bow and Oldman River basins and may have extended downstream into the upper Milk River basin (Fisheries and Oceans Canada 2014c). In the Oldman River drainage, 13 watersheds (35%) contain at least one genetically pure population, and in the Bow River drainage, eight watersheds (22%) contain at least one genetically pure population. The remaining populations are highly fragmented and occur in one or two streams, or a small portion of a stream. Many local extirpations of genetically pure populations have occurred in the historical range of the Westslope Cutthroat Trout including within the Bow River Drainage (Bow River below Lake Louise and the lower main stems of the Highwood, Elbow, Spray, Jumpingpound, Sheep, and Kananaskis rivers) and the Oldman River basin (Crowsnest River main stem). Historically, Westslope Cutthroat Trout was found in a variety of habitats, from headwater streams to main-stem river sections but, today, genetically pure Westslope Cutthroat Trout is mostly restricted to headwater streams and lakes and the upper reaches of mainstem rivers (Fisheries and Oceans Canada 2014c). Hybrid populations and those <99% pure still occur throughout most of the historical range.

Alberta Westslope Cutthroat Trout face several threats including competition, hybridization, introgression, and predation from non-native species, destruction of habitat resulting from resource extraction activities, and climate change (Rubidge et al. 2001; Taylor et al. 2003; Mayhood 2009; AWCTRT 2013; Yau and Taylor 2013). Rainbow Trout (*Oncorhynchus mykiss*), Rainbow Trout × Westslope Cutthroat Trout hybrids, and Yellowstone Cutthroat Trout (*O. c. bouvieri*) have been introduced throughout the native range of the Westslope Cutthroat Trout, leading to the loss of pure subpopulations via introgression throughout Alberta. Brook Trout, Brown Trout (*Salmo trutta*), and Lake Trout (*Salvelinus namaycush*) have also been introduced throughout the native range of Westslope Cutthroat Trout. These species often displace and replace native trout via competition and predation (Fisheries and Oceans Canada 2014c). Compounding the contemporary impacts of introduced species, Westslope Cutthroat Trout are expected to experience greater pressure from introduced species with climate change (Roberts et al. 2017; Muhlfeld et al. 2014, 2017). For example, Rainbow Trout has a higher thermal tolerance than Westslope Cutthroat Trout and a wider thermal tolerance range that overlaps the Westslope Cutthroat Trout (Bear et al. 2007), which has led to increased hybridization over time, even at cold-water sites (Muhlfeld et al. 2014, 2017). Similarly, Brook Trout tend to displace Westslope Cutthroat Trout because of a higher temperature tolerance and earlier age of maturity (COSEWIC 2016b). Given the interacting effects of non-native trout hybridization and global climate change, Westslope Cutthroat Trout is highly threatened by genetic extinction.

Although uncertain locations of pure genetic populations combined with the multitude of competitive and introgressive stocks of Westslope Cutthroat Trout makes recovery efforts challenging, reintroduction of extirpated populations and supplementation in small populations is recognized as a potential strategy that could help to protect the remaining pure populations (Fisheries and Oceans Canada 2014c; COSEWIC 2016b) (Table 1). Westslope Cutthroat Trout has been introduced within and outside its native range for over a century to replace or enhance local stocks, or to populate previously fishless lakes for recreational angling (COSEWIC 2016b). Due to the discontinuity between Westslope Cutthroat Trout subpopulations, genetic rescue is unlikely for

this species (Fisheries and Oceans Canada 2014c). Local translocations to supplement or re-establish populations have been considered as a potential action; however, care is needed when selecting source populations for reintroduction, as research has demonstrated that Westslope Cutthroat Trout populations display adaptations to local environmental conditions and may be maladapted for translocations (a characteristic of many salmonids) depending on the ecological characteristics of the recipient site (Drinan et al. 2012). In Alberta, Westslope Cutthroat Trout are raised in hatcheries (e.g., Sam Livingstone Hatchery, Calgary, Alberta) from annual egg samples collected from Job Lake, but these fish are only used to maintain the recreational fishery. Remote stream incubation is being tested in Alberta to determine its usefulness and practicality for reintroduction programs, but more genetic work needs to be done to ensure that the appropriate broodstock is used for each reintroduction event.

Like most imperilled fishes, conservation of Westslope Cutthroat Trout is challenging given the interactions among multiple stressors across the species range. An important ongoing first step is to identify, enumerate, and protect all the pure populations of Westslope Cutthroat Trout across the Canadian range (Fisheries and Oceans Canada 2014c, 2017d). Strategies for management of these populations will differ based on characteristics of individual systems, including the degree of hybridization in the population and presence of non-native trout species (Allendorf et al. 2001; Bohling 2016). For example, efforts are underway to protect pure Westslope Cutthroat Trout populations in Banff National Park by removing Rainbow Trout, Brook Trout, and their hybrids from the Cascade River watershed (AWCTRT 2013). This approach to management, namely the culling of invasive species, can only be effective when local stocks remain relatively protected from hybridization. For populations that show differing degrees of introgression, management becomes more complex (Allendorf et al. 2001; Bohling 2016). Preventing further introgression between species can be done by isolating target populations (e.g., Novinger and Rahel 2003; Muhlfeld et al. 2012) or by means of de-introgression approaches, which describes the process of recovering the genomes of populations impacted by adverse introgression (Amador et al. 2014). Efforts to isolate pure populations by installing barriers or removing non-native trout using mechanical or chemical methods must consider effects on other species at risk, such as Bull Trout (*Salvelinus confluentus*), which can co-occur with Westslope Cutthroat Trout. Further, recent research has used paleolimnological environmental DNA (eDNA) in sediments to determine the historical identity of stocked versus natural populations (Nelson-Chorney et al. 2019).

White Sturgeon—Lower Fraser River, Upper Fraser River, Upper Columbia River, Upper Kootenay River

White Sturgeon is one of the most iconic freshwater fish species in North America because of its unique morphology (cartilaginous skeleton and external bony scutes), long lifespan (>100 years), and large size (average adult TL = 120 cm; Scott and Crossman 1973). Unlike most freshwater SARA-listed species, the ecology and life history of White Sturgeon is relatively well-resolved (COSEWIC 2012c; Fisheries and Oceans Canada 2014d; Hildebrand et al. 2016). White Sturgeon is an opportunistic feeder that uses its protracile mouth to eat benthic invertebrates (e.g., crustaceans, mollusks) and fishes (e.g., smelt, salmonids; Billard and Lecointre 2001; COSEWIC 2012c). White Sturgeon prefers cool/cold waters (Coker et al. 2001) of large rivers, lakes, or reservoirs with adults typically occupying backwaters adjacent to eddies; however, this can vary based on the particular population and time of year (COSEWIC 2012c). In British Columbia, six populations of White Sturgeon exist that differ in geographic range, demographics, and genetics (Smith et al. 2002; Drauch Schreier et al. 2013): Lower Fraser, Middle Fraser, Upper Fraser, Nechako, Columbia, and Kootenay river

populations (Fisheries and Oceans Canada 2014d). Of the six, the Upper Fraser, Nechako, Columbia, and Kootenay river populations were previously listed as Endangered under SARA in 2006 (Fisheries and Oceans Canada 2014d). In November 2012, COSEWIC reassessed the species and the Middle Fraser, Nechako, and Upper Fraser river populations are now combined into a single Upper Fraser River DU. New, reorganized DUs of the Upper Fraser River (assessed by COSEWIC as Endangered in November 2012) and the Lower Fraser River (assessed by COSEWIC as Threatened in November 2012) are under consideration for listing under SARA (Table 1).

Threats to White Sturgeon populations differ based on the river system, but most are threatened by loss of habitat quality and quantity (including fragmentation due to dams, increases in turbidity, and altered thermal regimes), fishing, industrial activities, pollution, and overarching changes in ecological communities (Hatfield et al. 2004; Fisheries and Oceans Canada 2014d). As of 2012, there were an estimated 185 mature (>160 cm) White Sturgeon individuals in the Upper Fraser River, 243 in the Nechako River, 815 in the Kootenay River, and approximately 2579 in the Columbia River (Fisheries and Oceans Canada 2014d). The relatively low abundance of mature adults in the Upper Fraser River is thought to be within the natural range of variability for historical White Sturgeon populations (Ptolemy and Vennessland 2003). In the Nechako River, the White Sturgeon population is aging, with little to no recruitment observed since 1967 (McAdam et al. 2005; Fisheries and Oceans Canada 2014d). Extinction in this system is presumed inevitable unless human intervention can improve natural recruitment (Wood et al. 2007). Similarly, the status of the White Sturgeon populations in the Columbia River is deteriorating with natural recruitment in the wild being too low to sustain current population sizes (Fisheries and Oceans Canada 2014d). Likewise, the Kootenay River population has seen significant declines over the last several decades (Jager et al. 2010; Paragamian 2012), with the lowest degree of within-population genetic variability (Drauch Schreier et al. 2013). Although extirpations have not been observed for White Sturgeon within British Columbia, declining population trajectories and truncated age distributions have led to active population supplementation efforts through the release of hatchery-raised fish.

Population supplementation is proposed as a temporary, but long-term (potentially 40+ years) recovery strategy for White Sturgeon populations that has been ongoing in Canada for several decades (Fisheries and Oceans Canada 2014d; Table 1). Once stocked juveniles reach maturity, they are expected to eventually contribute to the spawning populations. Annual survival of hatchery-released White Sturgeon, estimated through mark-recapture studies, is very low in the first year but becomes substantially higher in subsequent years (88%–98%) and is dependent on the size at release (Gross et al. 2002; Irvine et al. 2007; Hildebrand et al. 2016). In the Kootenay River, approximately 284 000 individuals have been released since 1990 (Fisheries and Oceans Canada 2014d; Hildebrand et al. 2016). Furthermore, within-river translocations were initiated in 2003 and 2004 to move 25 mature sturgeon to areas more suitable for egg incubation and rearing (Rust 2011; Paragamian 2012). In the Nechako River, a total of 15 000 juveniles were released between 2006 and 2008, with more supplementation efforts expected with the opening of the Nechako White Sturgeon Conservation Centre in Vanderhoof, British Columbia (Fisheries and Oceans Canada 2014d). In the Columbia River, a hatchery program was initiated in 2001 and, by 2012, approximately 165 000 juveniles had been released into the Upper Columbia recovery area (Fisheries and Oceans Canada 2014d). Due to the aging populations and lack of recruitment across the White Sturgeon populations, captive-breeding efforts are expected to continue for the Kootenay, Nechako, and Columbia River populations.

Challenges for moving reintroduction efforts forward

This review of reintroduction efforts for Canada's SARA-listed fishes indicates that although many species have reintroduction identified as a recovery measure, only a handful of species (Atlantic Salmon, Atlantic Whitefish, Copper Redhorse, Striped Bass, and White Sturgeon; Table 1) have been the subject of reintroduction or enhancement programs for the purposes of conservation (Fig. 1). For several SARA-listed fishes (Channel Darter, Eastern Sand Darter, Gravel Chub, Lake Chubsucker, Northern Madtom, Pugnose Shiner, Redside Dace, Spring Cisco, and Westslope Cutthroat Trout), meaningful progress for conservation-based reintroduction initiatives has yet to occur. One reason for the slow progress is the relatively short time since SARA was enacted. Since 2002, for many species, conservation statuses needed to be assessed, SARA listing decisions made (many substantially delayed; Mooers et al. 2017), management plans, recovery strategies, and action plans developed and implemented, including research in support of reintroduction. In other cases, species earmarked for reintroduction were listed well after the Act came into force. Since enactment, most research for SARA-listed species focused on basic aspects of ecology rather than reintroduction per se. As well, given the lack of direct commercial or recreational value, many SARA-listed species (particularly small-bodied fishes) have been neglected in the research domain and lack stakeholder support. Nevertheless, the past 16 years of research on SARA-listed species has addressed numerous information gaps and many of these will have strong bearing on the design of future reintroduction programs.

Despite the relative scarcity of reintroduction studies for freshwater organisms compared to terrestrial organisms (Seddon et al. 2005; Champagnon et al. 2012), there are examples of reintroduction for imperilled fishes not listed for protection under SARA and a long history of introductions for recreationally and commercially important fishes that can provide guidance for future reintroduction efforts of SARA-listed species. For example, reintroduction was performed in Canada for American Eel (*Anguilla rostrata*; Pratt and Threder 2011; Verreault et al. 2010), Atlantic Salmon in Lake Ontario (Stewart and Schaner 2002; Stanfield and Jones 2003), Aurora Trout (*Salvelinus fontinalis timagamiensis*; Snucins et al. 1995), and Lake Trout in Lake Ontario (Elrod et al. 1995) and many formerly acidified southcentral Ontario lakes (Keller et al. 1990). Greater progress has been made in the United States than Canada in developing artificial rearing approaches and evaluating the effectiveness of translocations. Many imperilled freshwater fishes in the United States have been successfully reintroduced, such as Duskytail Darter (*Etheostoma percnurum*), Smoky Madtom, Spottfin Chub (*Erimonax monachus*), and Yellowfin Madtom in Tennessee (Shute et al. 2005), Lake Sturgeon (*Acipenser fulvescens*) in the Big Manistee River in Michigan (Holtgren et al. 2007), the Mississippi and Missouri rivers (Drauch and Rhodes 2007), and Oneida Lake (Jackson et al. 2002), and Bonytail (*Gila elegans*), Flannelmouth Sucker (*Catostomus latipinnis*), and Razorback Sucker (*Xyrauchen texanus*) in the Lower Colorado River (Mueller 2003; Mueller and Wydoski 2004; Schooley and Marsh 2007). In the above examples, even basic information gained about aspects of population ecology and genetics, habitat science, and threat science prior to initiating management actions was informative from a reintroduction perspective.

To encourage greater research efforts to inform fish reintroductions in Canada, we outline how basic research questions concerning population ecology, habitat science, and threat science can provide a critical foundation for addressing knowledge gaps in reintroduction science. With this, we provide broadly applicable reintroduction questions that, if addressed, will contribute significantly to the success of reintroduction programs for SARA-listed species (Table 2). These four themes (population ecology, habitat science, threat science, reintroduction science) were identified by

Canadian aquatic scientists and managers as important research areas needed to support recovery activities for freshwater fishes and mussels in Canada (Drake et al., unpublished data). We follow these summaries with a section regarding the importance of genetic and genomic techniques for informing these research questions. Finally, we close with a perspective on engaging in active, experimental approaches for moving reintroduction efforts forward in Canada.

Population ecology

Data limitations around basic species-specific attributes of SARA-listed fishes can slow the progress of conservation initiatives, as some degree of scientific certainty is required before undertaking relocation projects, particularly for efforts that could negatively impact source or recipient populations/communities (e.g., translocations, supplementation). Such data limitations include knowledge of abundance, distribution, genetic structure, life-history characteristics, reproductive biology, and species interactions (Table 2). In addition, population vital rates (i.e., estimates of growth, immigration, mortality, reproduction, and survival) are rarely known with certainty for imperilled species, but are useful for developing population models and performing population viability analyses (PVAs; Morris et al. 2002). Population models, including PVAs, provide an opportunity to assess risk for imperilled populations by projecting trajectories of species abundance over time, therefore providing tangible targets for species protection and recovery. However, numerical targets alone can lead to a false sense of recovery and, therefore, should represent only one component of the recovery criteria (Wolf et al. 2015).

Existing PVAs often identify early life stages as the most sensitive to environmental stressors or catastrophic events (e.g., Young and Koops 2011; Finch et al. 2017); therefore, a better understanding of early life stage ecology and survival among extant populations would be particularly useful for planning reintroduction activities. However, studies on this part of the life cycle for many SARA-listed species are difficult to undertake and are rarely the first to be conducted. Locating spawning and nursery habitats and, thus capturing juvenile fishes, can be challenging for imperilled species. Recent developments in eDNA sampling approaches, which are used to detect species based on DNA released into the aquatic ecosystem (Balasingham et al. 2018), may help to resolve this problem (Jerde et al. 2011; Thomsen et al. 2012; Janosik and Johnston 2015). Targeted eDNA sampling in potential spawning habitats during periods in which spawning is thought to occur can provide direction for further investigation (e.g., targeted larval sampling). For example, once spawning areas are identified, mark-recapture studies or site-specific monitoring can be initiated to determine local population vital rates for better informing PVAs leading to tangible targets for reintroduction purposes. As well, targeted sampling efforts can provide insight into species mating systems and reproductive attributes, providing information as to whether random mating is suitable for captive-breeding efforts. Nevertheless, as a result of rarity in most SARA-listed species, targeted sampling of extant locations may only provide information on a small number of individuals.

Habitat science

Species reintroduction efforts will not be successful unless sufficient habitat quality and quantity exist at relocation sites. Ensuring that sufficient habitat is available requires a thorough understanding of species-habitat associations across life stages (Lamothe and Drake 2019). However, for several SARA-listed species, such as the Eastern Sand Darter and Northern Madtom, less is known about juvenile life-stage habitat associations than for adults. This is primarily due to the difficulty of collecting and identifying larval and juvenile fishes for species already at low population abundance and with reduced ranges; the species reviewed in this paper show geographically discontinuous distribu-

Table 2. Relevant questions to the reintroduction of *Species at Risk Act* listed fishes, organized by conservation research themes and sub themes.

Research theme	Research sub theme	Questions
Population ecology	Life history	Are population vital rates (fertility (both sexes), mortality, growth/age structure, immigration, emigration) known?
		Do population bottlenecks exist? If so, which life stages are involved?
	Abundance	Is reproductive biology well understood (e.g., age at maturity, spawning cues, mate choice, Balon guild)?
		What are population sizes in the wild for potential source stocks or newly introduced populations?
	Distribution	What is the spatial configuration of extant (source) populations?
	Genetic structure	Is recolonization through natural migration likely to occur?
What is the genetic structure within and across populations?		
Species interactions	Are there known obligate, facultative, or parasitic species dependencies?	
	What are the effects of reintroductions or translocations on the existing fish community and ecosystem (including pathogens and parasites)?	
Habitat science	Behaviour	What are the effects of invasive species on the imperilled species and its recovery?
		How do translocations or captive breeding efforts alter species behaviour?
	Species habitat associations	What are critical habitat requirements across life stages?
Habitat supply		Which environmental conditions (abiotic and biotic features) promote successful recruitment?
Threat science	Mechanism of impact	What were the historical habitat conditions for each species locality?
		Do present conditions reflect historical conditions?
		How much habitat is needed to support a viable population?
Recovery science	Threat mitigation	Is habitat supply sufficient in Canada to support reintroduction?
		How much variation exists in ecosystem conditions across the species' range?
		What factors caused the original extirpation, and have they been eliminated or reduced?
Recovery science	Reintroductions	Have new threats occurred since the species was extirpated and if so, how will the species respond to those threats?
		Is the repatriation site secure from future threats?
		How will climate change impact potential reintroduction sites?
		How long after threat mitigation should reintroductions be undertaken?
		What is the likelihood of restoration activities achieving historical habitat conditions?
		Which ecological factors are most important when selecting a source stock (e.g., population size, genetic relatedness, proximity to reintroduction site)?
		Are procedures for captive breeding and rearing established for the species?
		Should the translocation of wild fish or the release of captive-reared individuals be used in reintroduction efforts?
		How does stocking frequency, timing, number, and spatial distribution impact the probability of persistence?
		How does the relationship between individuals stocked and the likelihood of natural reproduction in the wild vary among stocked individuals (e.g., age/maturity, condition, time of year)?
		What are the risks and benefits of species relocation outside the historical range, both for target species and other ecosystem components?
		What are the risks/benefits of Ark populations, and where should they be located?
What are first-year survival rates for stocked individuals?		
When does the first reproductive event occur post-reintroduction?		
Which non-invasive tools exist to mark stocked individuals for follow-up monitoring requirements?		
How should the reintroduction be set up in an appropriate experimental design?		
How should monitoring efforts be designed to evaluate a species reintroduction program (e.g., success of new recruits, habitat changes)?		
How should genetic tools be used to guide monitoring design?		

Note: Reintroductions are part of the recovery science research theme; however, questions relevant to reintroductions span on all four research themes.

tions across Canada, often with low local abundance. Adding to the issues of rarity, sampling SARA-listed species often requires the development of species-specific sampling approaches due to unique habitats occupied, and the effects of these sampling efforts on the habitat and population itself should be considered (Dextrase et al. 2014b). For example, Lake Chubsucker tends to primarily inhabit heavily vegetated areas of floodplain lakes, marshes, and wetlands (Mandrak and Crossman 1996; COSEWIC 2008; Staton et al. 2010). Electrofishing and seining are both inefficient for fishes in these habitats, particularly during times when vegetation density is high (e.g., spawning season). These sampling challenges make it difficult to answer basic questions that will nonetheless inform reintroduction outcomes, such as “what are

critical habitat requirements across life stages?” (Table 2), especially if juvenile habitat is a bottleneck to recovery. Despite these challenges, it is important that targeted sampling approaches with ongoing methodological development (e.g., integrated population-habitat models) for SARA-listed fishes continue to better inform our knowledge of SARA-listed species-habitat relationships as it relates to future reintroductions.

Threat science

Identifying the causative factors that led to species extirpation should be the first research priority before considering reintroduction (Armstrong and Seddon 2008; Cochran-Biederman et al. 2015). However, knowledge of historical conditions and threats is

often lacking, making it difficult to establish historical baselines and definitively identify the causes of extirpation. In such cases, the best possible information may come from comparisons of present-day habitat characteristics (e.g., water quality, substrate composition) and community composition between potential reintroduction sites and areas where the species remains extant (Harig and Fausch 2002; Schadt et al. 2002; Dextrase et al. 2014a).

While ensuring that the original cause of extirpation is mitigated is a priority, so too is framing future reintroduction efforts with a global climate-change lens (Jones et al. 2016). That is, what is the probability, extent, and magnitude of potential impacts from climate change at reintroduction sites (Table 2)? Several populations of SARA-listed species described in this review are at the northern extent of their range (e.g., Westslope Cutthroat Trout), which makes them particularly vulnerable to climate change given that several fish species at the northern extent of their range have demonstrated range contractions over the last several decades (e.g., Alofs et al. 2014). Species distribution modelling can be used to predict changes in species distributions with varying climate scenarios and, ultimately, help identify suitable sites for reintroduction (Olden and Jackson 2002; Hernandez et al. 2006; Thuiller et al. 2009). The most direct way to understand the consequence of particular threats on species distributions and persistence is through experimental trials, whether in the laboratory or through translocations or reintroductions from captive-breeding programs. By performing experimental trials in a systematic, structured framework to allow hypothesis development and testing, many questions related to threat science (e.g., Table 2) can be answered to help inform reintroduction efforts.

Recovery science

Reintroduction represents one commonly cited approach for the recovery of SARA-listed species (Table 2). Guidelines for preparing and conducting species reintroductions have been developed for wildlife species in general (IUCN/SSC 2013) and specifically for fishes (Williams et al. 1988; George et al. 2009). Furthermore, a Canadian code on species introductions and transfers of aquatic organisms is available (Fisheries and Oceans Canada 2017e). Prior to undertaking reintroduction, threat elimination or mitigation is required. After threats have been eliminated or mitigated, and where suitable habitat is present, reintroductions can be performed via translocation or release from propagation efforts (i.e., captive breeding, stream-side rearing). For several species described in this review (e.g., Spring Cisco, Copper Redhorse, Atlantic Whitefish), only a single stock for captive breeding exists; however, for other species, identifying which source stocks to use is more challenging. More research is needed to understand the implications of removing individuals from local populations to seed reintroduction efforts in Canada, particularly for smaller-bodied species like Eastern Sand Darter, Lake Chubsucker, Pugnose Shiner, and Redside Dace, or when genetics and local subpopulation adaptations are a factor, such as for Westslope Cutthroat Trout. Translocation or captive breeding of small-bodied fishes having known or suspected high maximum per capita growth rates (r) is rare compared to larger-bodied, longer-lived fishes, but species with a life-history strategy that yields a relatively high r_{\max} (i.e., $r_{\max} > 1$; Hutchings et al. 2012) may require fewer introductions (George et al. 2009). In addition, questions related to how to identify the best source stocks when weighing genetic versus ecological similarity is a top priority for potential species translocation efforts. Finally, evaluation of the risks of pathogen propagation (i.e., viruses, bacteria, parasites) must also be considered during the planning stages of repatriation efforts (Table 2).

To date, there have been captive-breeding programs for only a handful of SARA-listed freshwater fishes in Canada for the purposes of conservation, including Atlantic Salmon, Atlantic Whitefish, Copper Redhorse, Striped Bass, and White Sturgeon (Table 1).

The experiences gained from these efforts to recover populations, both in terms of successes and failures, will provide a structured path forward for reintroduction efforts of the other species. For example, the knowledge gained through conservation efforts of iBoF Atlantic Salmon populations has arguably led to the greatest knowledge advancements for any species in Canada, imperilled or otherwise. This knowledge was not available at the start of the Atlantic Salmon Live Gene Bank Program in 1998, rather, knowledge and understanding developed as scientists and managers acted to make the best evidence-based decisions for protecting the species. However, the experience with Atlantic Salmon also illustrates an important challenge for species conservation in that the success of reintroductions can be hampered by unknown ecological factors (e.g., the cause of high mortality at sea). Nevertheless, while these experiences of captive breeding and subsequent release provide valuable experience for future conservation-based captive-breeding programs, initiating breeding programs for the other species in this review will present their own ecological (e.g., establishing a gene bank, maintaining genetic diversity) and operational challenges (e.g., establishing long-term funding to support species monitoring requirements, disease).

Genetics/genomics aspects of reintroduction for SARA-listed fishes

Reintroduction efforts involve the movement of genes into a historical part of a species range, which can alter the genetic structure of the source and reintroduced populations. As such, those charged with reintroduction efforts must make many decisions that will affect the genetic makeup of the released (and source) populations and these effects may last for generations (reviewed in Weeks et al. 2011; Keller et al. 2012). Until recently, assessment of the genetic makeup of source or reintroduced populations was conducted using traditional genetic techniques (e.g., microsatellites for population genetics; Ozer and Ashley 2013; Geneva et al. 2018). Present-day genomic technologies can now be used to augment the power of population genetics using additional markers (e.g., hundreds or thousands of single nucleotide polymorphisms (SNPs)) to cover more of the genome (reviewed in Allendorf et al. 2010; Ouborg et al. 2010; Oomen and Hutchings 2017; Connon et al. 2018). These techniques have also been used in a paleolimnological context to determine historical biogeography of SARA-listed species (Nelson-Chorney et al. 2019), which can help elucidate areas previously occupied by a species for targeted reintroduction. In addition, transcriptomics is now being explored to assess fitness-related genetic variation, measuring how environmental stressors affect gene activity, and determining the molecular mechanisms of tolerance to environmental stressors (reviewed in Connon et al. 2018). As such, genetic and genomic considerations are required to address several challenges for reintroduction including: managing issues related to the small populations, determining source populations for reintroduction, designing captive-breeding programs, and for post-release monitoring. Below, we highlight some of these fundamental genetic/genomic considerations related to reintroduction efforts for SARA-listed fishes.

Reintroduced populations are small

Reintroduced populations are typically derived from small populations that have experienced a reduction in population size for an extended period. As a result, reintroduced populations may suffer from the same potentially deleterious issues as small populations including Allee effects, genetic drift, loss of evolutionary potential (i.e., adaptive potential), and inbreeding (Frankham et al. 2010; Allendorf et al. 2012; Hutchings 2015). Random genetic fluctuations from one generation to the next, known as genetic drift, become more pronounced the smaller the size of the population. Potentially deleterious long-term consequences include in-

creased frequency of deleterious mutations, reduced adaptive evolutionary potential, and inbreeding depression. These negative outcomes often occur because genetic drift leads to a loss of genetic variation, with rare alleles being lost faster than common ones resulting in a decline in population level heterozygosity. Effective population size is often used as a proxy for measuring genetic drift by estimating the change of allele frequencies at a set of loci over several generations, most commonly using microsatellites (e.g., Hutchinson et al. 2003). However, in the last decade, conservation studies have moved away from genotyping microsatellites at tens of loci to quantifying variation at thousands of SNPs across the entire genome (e.g., Pazmino et al. 2017).

Because environmental stressors are not constant, organisms often use plasticity to cope with varying degrees of success. In the absence of phenotypic plasticity (or going beyond its scope), adapting to changing environmental stressors requires adaptive evolutionary change; therefore, part of the challenge of conserving reintroduced populations is to maintain necessary raw material for adaptive evolutionary change in response to selective challenges in their environment—known as evolutionary potential or adaptive potential (Frankham et al. 2010). The raw material for evolutionary potential in reintroduced populations is genetic variation, with higher variation presumably increasing evolutionary potential (Franklin 1980; Caballero and Garcia-Dorado 2013; but see Neff et al. 2011 for a discussion of preserving genetic adaptations rather than only genetic diversity). Estimating evolutionary potential is difficult because it is related to additive genetic variance of traits under selection, which can only be estimated effectively and accurately using laborious and expensive quantitative genetics experiments (see Lynch and Walsh 1998). For example, Houde et al. (2015a) conducted full-factorial quantitative genetic breeding design experiments on three Atlantic Salmon populations being used for reintroduction efforts in Lake Ontario to assess additive genetic variance in juvenile survival and growth (see also Houde and Pitcher 2016). Molecular methods can also be used to estimate evolutionary potential; however, a handful of microsatellites is not considered sufficient. With the development of a larger number of markers for conservation studies via next-generation sequencing techniques, the estimation of additive genetic variance will presumably improve substantially.

Reintroduced populations are typically small and, as a result, individuals will often have little choice but to mate with relatives. Inbreeding in reintroduced populations may be unavoidable simply due to small pool of breeding individuals (Keller et al. 2012). In most species, inbreeding has harmful effects on fertility, survival, and other fitness related metrics—resulting in inbreeding depression (Charlesworth and Willis 2009). Inbreeding depression arises, in part, because inbred mating often exposes numerous mildly deleterious recessive alleles that become expressed in homozygous forms in inbred individuals. Levels of potential inbreeding and inbreeding depression can be measured using a combination of pedigrees (when available) and genetic markers. Specific loci to examine inbreeding depression (especially those with large effects) can be found using several approaches including candidate loci, homozygosity mapping, or genome-wide associate studies (see Hedrick and Garcia-Dorado 2016). For SARA-listed species, pedigrees are rarely available so there is more of a reliance on genetic markers to assess levels of relatedness within the population.

Source-population selection

Under ideal circumstances, the source population(s) to be used for reintroduction efforts would possess substantial evolutionary genetic potential to ensure the future viability of the population and avoid deleterious factors such as inbreeding or outbreeding depression. Houde et al. (2015b) developed a source population selection framework (when multiple populations sources are available), which includes sourcing a population from another

that has a similar environment (“environmental matching”) and two approaches that require genetic evaluation. First, the “ancestry matching” strategy suggests that source populations differ in their likelihood of establishment based on differences in genetic parameters related to fitness due to local adaptation. The optimal source population for this strategy is selected based on genetic similarity to the extirpated population under the assumption that genetic relatives of the original population share genes that offer survival and (or) reproductive advantages. This strategy therefore requires knowledge regarding the genetic similarity between the population that is extirpated and the possible source populations. For example, to examine potential source populations for the Endangered Redside Dace, Serrao et al. (2018) characterized the genetic structure and diversity across the species range using mitochondrial and microsatellite data.

The second genetically based option, “adaptive potential”, is to choose among source populations based on the potential for those individuals to adapt to key environmental stressors found at the new location. This strategy therefore favours the reintroduction of source populations with high heritable genetic variation. The adaptive potential strategy can be accomplished by using either a single source population with high heritable genetic variation, measured using quantitative genetic breeding designs with an emphasis on key fitness-related traits or, to a lesser extent, using neutral genetic markers to estimate features such as heterozygosity (which is assumed to be correlated with fitness-related metrics). An alternative option for this strategy is to use multiple source populations that are genetically dissimilar to each other in the hopes that one of the populations possess genetic variation ideal for the new location (i.e., a genetic bet-hedging strategy). This strategy does require the recognition that there is the potential for outbreeding depression between distant populations of the focal species when multiple source populations are used, which can often manifest in second or third generations of outbreeding. For example, outbreeding depression was detected in Slimy Sculpin (*Cottus cognatus*) when reintroduced to part of its historical range (Huff et al. 2011; but also see Audet et al. 2017).

Transcriptomics may provide new avenues for selecting populations for reintroduction of SARA-listed fishes. Transcriptomics, which examines messenger RNA, may allow for a more thorough understanding of mechanisms related to the tolerance or acclimatization of species to environmental change (see Oomen and Hutchings 2017; Connon et al. 2018). For example, a transcriptomic approach was used in a source population selection exercise for the reintroduction of extirpated Atlantic Salmon into Lake Ontario, Canada (He et al. 2015). Using a custom microarray, significant gene transcription differences were found at 21 genes between two possible source populations, demonstrating that differences are likely the result of selection, and a source population was recommended for reintroduction based on those differences (He et al. 2015, 2017).

Captive breeding

Wild source populations are not always available for reintroduction efforts. In these cases, individuals from captive-breeding programs can be reintroduced into the wild. Captive breeding faces the same general issues that face small populations, including consequences of genetic drift, loss of genetic variation, increased inbreeding, and possibly inbreeding depression. The most recognized consideration of genetics for captive breeding for reintroduction purposes is minimizing inbreeding and maximizing the maintenance of genetic diversity in captivity. For example, Aurora Trout was extirpated from the wild during the 1960s because of lake acidification in northern Ontario (Snucins et al. 1995). However, these fish escaped extinction, thanks to a captive-breeding program founded with the last nine surviving individuals, which allowed the trout to persist in captivity and eventually

be reintroduced to their native habitat where natural reproduction and recruitment were eventually observed. Neff et al. (2011) suggested that captive-breeding programs should be used only as a last resort when populations face imminent extirpation and that such programs must shift the focus from solely preserving genetic diversity to preserving genetic adaptations.

One of the key genetic processes that differ drastically between captive-bred and wild-origin populations is selection. Selective pressures differ between captive and natural environments, often leading to relaxed selection against deleterious alleles in captivity and to adaptation to captivity (see Fraser 2008). For example, life-history traits in Chinook Salmon (*Oncorhynchus tshawytscha*) (e.g., egg size) were unintentionally selected to be smaller in captive-rearing settings (i.e., hatcheries) compared to their wild-origin counterparts (Heath et al. 2003). As well, rearing environment and techniques may induce selection for maladaptive behaviours (e.g., inability to recognize food or avoid predation) is commonly reported for individuals bred and (or) reared in captivity (Johnsson et al. 2014; Wilke et al. 2015; Tave et al. 2018). These kinds of captivity-based selection effects can be detrimental for reintroduction success and may help explain the relatively poor fitness of some captive- versus wild-origin individuals. These captivity-based maladaptive selection effects are often a function of duration in captivity and effective population size (Fraser 2008); therefore, one of the key mechanisms to avoid captive selection effects is to minimize time individuals spend in captivity before release or attempt to make the captive environment more “natural”. Neff et al. (2011) suggested that incorporating as many aspects of natural ecological processes as possible into captive-breeding programs would help improve the fitness of offspring produced. For example, sexual selection could be incorporated into captive-breeding programs by facilitating mate choice and male-male competition (see Pitcher and Neff 2007). Features of natural selection such as disease challenges and predation could also be incorporated to minimize selection imposed by the artificial captive-breeding environment and to promote genes associated with local adaptation to the natural environment.

Post-release monitoring

One way to assay the success of a reintroduction effort is to examine patterns of neutral and adaptive genetic variation of the reintroduced population over the course of several generations. To do this, genetic markers (e.g., microsatellites, SNPs) can be examined for significant changes in frequency over multiple generations to identify outlier loci that exhibit evidence for divergent selection as opposed to drift. For example, Campbell et al. (2017) used restriction-site associated DNA sequencing to genotype 5392 SNPs in reintroduced Coho Salmon (*Oncorhynchus kisutch*; released in three separate years) to examine neutral and potentially adaptive genetic variation over four generations and found that there was little gene flow between the reintroduced brood populations suggesting it may take quite some time for reintroduced alleles to completely permeate the new populations being created.

Conclusion

Species reintroduction represents an important component of a multi-tiered species conservation program and considerable information about the species and local environment is desirable to ensure success. Although less is known about the reviewed species than commercially or recreationally important species, our review demonstrates that reasonable progress in addressing basic ecological questions for SARA-listed species has been made. When planning reintroductions and translocations for recovery with the goal of down-listing conservation status, the COSEWIC guidelines for manipulated populations must be considered (COSEWIC 2010c). These guidelines outline under what conditions reintroduced or translocated individuals should be included when applying quantitative criteria to determine conservation status. In

general, individuals or reintroduced populations within the native range of a species or DU should be included, whereas introductions outside of the native range should be excluded unless suitable habitat no longer exists within the native range.

As imperilled fishes become increasingly rare, implementing conservation and protection approaches becomes more difficult. With fewer individuals in the wild to study, there are fewer opportunities to identify important biological characteristics that will aid recovery. The expectation for complete scientific certainty covering all relevant research questions (e.g., population ecology, habitat science, threat science) for SARA-listed species is unrealistic over relatively short timeframes. Delaying reintroduction efforts to obtain this information may lead to undesirable trade-offs, such as the need to implement conservation actions for longer timeframes and with greater resources (Naujokaitis-Lewis et al. 2018) and may jeopardize the recovery of a species when abundance is low and sampling becomes difficult. Given the immediate need for protection of SARA-listed species and the uncertainty surrounding fish species reintroduction, the best approach for moving reintroduction forward lies within an adaptive management framework, where experimentation is used to optimize management decisions (McDonald-Madden et al. 2011; McCarthy et al. 2012; Sard et al. 2016; West et al. 2017).

Adaptive management has a long history in fisheries management (Walters and Hilborn 1976, 1978; Holling 1978). By clearly stating objectives, communicating hypotheses, developing models, designing experiments, implementing monitoring programs, and applying management actions within a reintroduction context, and iteratively learning from these efforts, knowledge gaps can be reduced (e.g., Table 2) while actively engaging in species recovery efforts. For species where reintroduction efforts have occurred (e.g., Copper Redhorse, Atlantic Salmon), species persistence can largely be attributed to efforts by scientists and managers engaged in active, adaptive management, whereby management actions were taken, successes evaluated, and lessons learned were applied to the next iteration of management actions. However, due to practical and logistical constraints (e.g., low supply of brood stock, insufficient resources to simultaneously undertake multiple reintroductions, poor resources for long-term follow-up monitoring), most reintroduction efforts in Canada have not been performed with a structured experimental design that involves applying alternative management scenarios, thereby limiting our ability to draw conclusions from such efforts. Although some reintroduction experiments will ultimately fail, if an adaptive management approach is carefully executed, the results will provide useful information that will ultimately help to improve species recovery.

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