



## EVALUATING THE ECOLOGICAL BENEFITS, RISKS, AND FEASIBILITY OF REINTRODUCTION FOR EASTERN SAND DARTER (*AMMOCRYPTA PELLUCIDA*) IN ONTARIO



Figure 1. Eastern Sand Darter (*Ammocrypta pellucida*) from the Grand River, Ontario, Canada. Photograph by DFO (Karl A. Lamothe).

### CONTEXT

Federal recovery strategies frequently identify species reintroduction as a potential approach to improve the survival and recovery of species listed under the Species at Risk Act (SARA), including for Eastern Sand Darter (*Ammocrypta pellucida*). However, there has been a lack of reintroduction effort over the last two decades, in part due to incomplete knowledge of the ecological requirements of the species and uncertainty about how to assess the ecological benefits, risks, and feasibility of reintroduction. Over the last 15 years, understanding of Eastern Sand Darter ecology has improved and recent national guidance describes the scenarios in which reintroduction could be considered as a recovery tool, which together provide an opportunity to evaluate the use of reintroduction to achieve species recovery targets. Three objectives were addressed:

1. Evaluate ecological factors that could influence successful reintroduction of Eastern Sand Darter in candidate recipient and source locations;
2. Evaluate the ecological risks associated with reintroduction of Eastern Sand Darter, both for the species and broader ecosystem at source and recipient locations; and,
3. Evaluate the change in survival or recovery of Eastern Sand Darter resulting from the re-establishment of a population in a formerly occupied location.

The advice generated from this meeting can be used by the Species at Risk Program to determine if the benefits of an experimental reintroduction outweigh the potential risks for Eastern Sand Darter and the broader ecosystem, and can serve as a case study when considering reintroduction for other SARA-listed freshwater fishes.

This Science Advisory Report is from the April 3–6, 2023 Regional Peer Review meeting on the Evaluating the Ecological Benefits and Risks of an Experimental Eastern Sand Darter (*Ammocrypta pellucida*) Reintroduction in Ontario. Additional publications from this meeting will be posted on the [Fisheries and Oceans Canada \(DFO\) Science Advisory Schedule](#) as they become available.

## SUMMARY

- Reintroduction is identified as a potential recovery measure for the Ontario designatable unit of Eastern Sand Darter (Figure 1; *Ammocrypta pellucida*; listed Threatened under the *Species at Risk Act*) due to extirpations from three formerly occupied watersheds. There is uncertainty about how reintroduction could change the survival or recovery of the wildlife species (hereafter species), as well as the ecological feasibility and risk of undertaking translocations from wild populations.
- The implications of several translocation scenarios for the probability of species extirpation were considered. The successful addition of a reintroduced population lowered species extirpation probability.
- Models were developed to evaluate potential abundances in source populations, the effect of removing individuals from source populations, and the potential for population establishment in recipient locations. Habitat suitability and threats were also described for recipient locations.
- The Grand and Thames rivers were considered as potential sources, with model predictions suggesting that Eastern Sand Darter abundance is potentially higher in the Grand River than the Thames River. Model outputs were highly dependent on the assumed availability of suitable habitat and density of fish in waters deeper than 1.2 m.
- Abundance and the probability of catastrophic events were important variables for predicting harm from removals. Source rivers with higher abundance were better able to withstand removals; however, even without removals, source abundances can fluctuate and fall below thresholds for harm.
- The total number of individuals released and the carrying capacity in recipient rivers were important variables for predicting reintroduction success; success was most likely to occur with a larger number of additions and a higher carrying capacity in recipient rivers.
- Sand and fine gravel substrate, as well as water clarity, were previously identified as important factors for the occurrence and abundance of Eastern Sand Darter, with predicted occupancy of Eastern Sand Darter in the Ausable River and Big Otter Creek. Substrate sampling at potential release sites in Big Otter Creek suggest relatively high proportional cover of sand and fine gravel in areas where Eastern Sand Darter previously occurred.
- The effects of agriculture and Round Goby (*Neogobius melanostomus*) invasion were identified as important threats that may influence the successful reintroduction of Eastern Sand Darter in potential recipient rivers, but these threats also occur where the species is extant and in good condition. Cumulative threats were not assessed. Disease was also identified as a potentially important consideration.

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- Model outputs and the broader evidence base were considered in a structured expert judgement process (modified Delphi) to evaluate factors related to the overall ecological feasibility and risk of unintended ecological consequences of translocation.
- Structured expert judgements were generally consistent with model outputs, but experts indicated substantial uncertainty about most factors associated with the ecological feasibility of translocation. Generally, there was greater agreement that: 1) the Grand River could likely withstand low levels of removal (250 fish per year for up to 10 years), and 2) life history, genetic factors, competition/predation, and current threats (Round Goby, agriculture) would likely not impede establishment in recipient locations. The availability of habitat and food supply to support larger abundances of fish in recipient rivers had less agreement, though these factors were unlikely to impede smaller numbers of fish from establishing, with slightly greater confidence around the habitat suitability and availability in Big Otter Creek.
- The risks of unintended ecological consequences of translocation to source and recipient rivers were deemed to be generally low to moderate by experts, but with some uncertainty. Genetic risks (inbreeding depression, outbreeding depression, founder effects) were of relatively low concern given the proposed experimental reintroduction, but were contingent on where and how fish were collected from source rivers. Transformational ecosystem changes were considered unlikely to occur. The potential for disease introduction was deemed to be low, but if introduced, novel pathogens could have significant impacts.
- Generally, expert judgements were less certain on factors related to ecological feasibility, including source abundance, the effect of source removals, and abiotic and biotic habitat availability, and were more certain on factors related to the risk of unintended ecological outcomes in source and recipient ecosystems. However, the expert perceptions of potential introduction and ecological consequence of disease had higher uncertainty.

### **BACKGROUND**

Eastern Sand Darter (*Ammocrypta pellucida*) is a small freshwater fish species listed for protection under the Species at Risk Act (SARA). In support of the long-term recovery objective of restoring self-sustaining populations to formerly occupied habitats, the federal recovery strategy for the Ontario DU identified the need to investigate the feasibility of reintroduction (DFO 2012). Reintroduction efforts have yet to occur for Eastern Sand Darter, at least in part due to incomplete knowledge of the ecological requirements for the species and uncertainty about how to assess the ecological benefits, risks, and feasibility of reintroduction. A national Canadian Science Advisory Secretariat (CSAS) process recently developed a decision support framework to provide guidance around scenarios where reintroduction could be considered as a recovery tool for SARA-listed freshwater fish and mussel species (Figure 2; DFO 2023). Application of this framework provides decision makers with the ecological information about how reintroduction could benefit a particular SARA-listed species, and the ecological feasibility and risks of performing such an action. Understanding of Eastern Sand Darter ecology and the conditions at currently and historically occupied locations has improved greatly over the last 15 years, making it an ideal candidate species to evaluate reintroduction feasibility. The five-step decision support framework was used to evaluate the benefits, risks, and feasibility of an Eastern Sand Darter reintroduction in southwestern Ontario. This document provides a review of Steps 1–3 (described in Lamothe et al. 2023), and describes certain components of Step 4 that were completed during the CSAS meeting.

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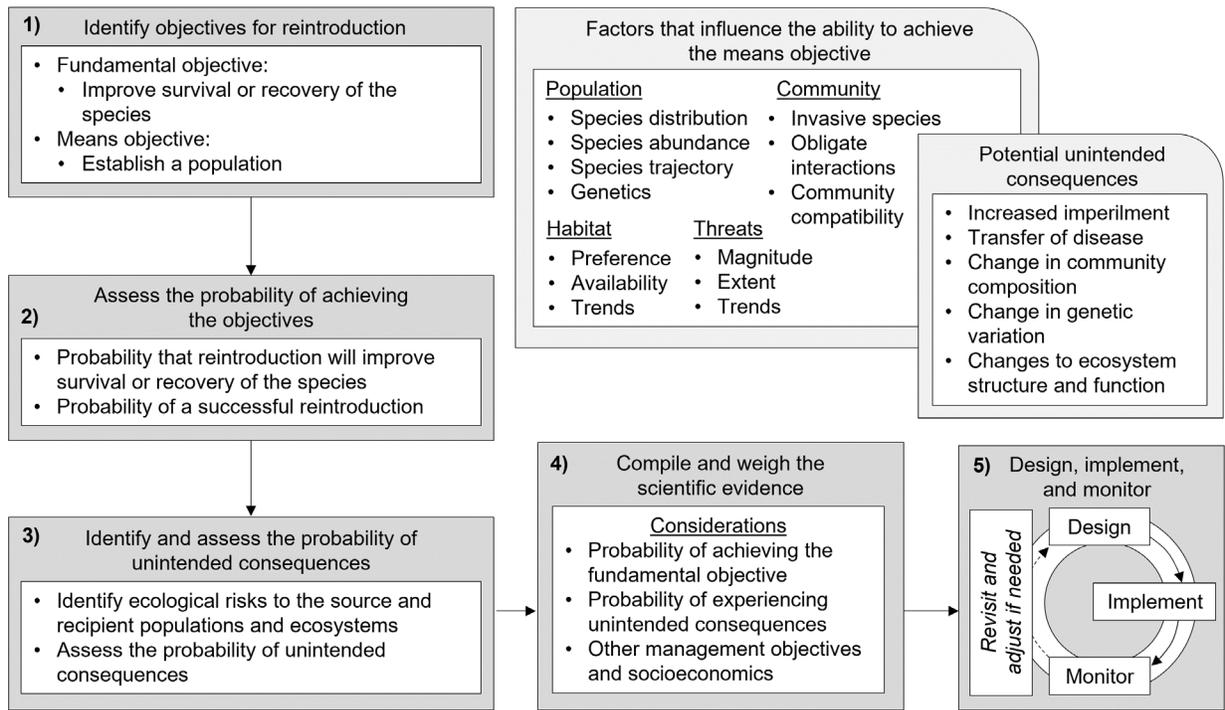


Figure 2. Decision support framework outlining science considerations for the use of reintroduction as a tool to improve the survival or recovery of freshwater species listed under the Species at Risk Act. Modified from Lamothe et al. (2023).

## ASSESSMENT

### Step 1 – Identify the objectives of reintroduction and the focal populations

The first step in considering reintroduction for Eastern Sand Darter is to develop a problem statement and identify the fundamental and means objectives of performing the management action (Figure 2). Here, the fundamental and means objectives were to improve the survival or recovery of Eastern Sand Darter in southwestern Ontario by means of reintroduction. Eastern Sand Darter has been extirpated in at least three waterbodies in southwestern Ontario: Ausable River, Big Otter Creek, and Catfish Creek. Only the Ausable River and Big Otter Creek were considered as potential recipient locations as there was poor data availability for Catfish Creek, and initial judgements indicated Catfish Creek to be unsuitable for the species.

There are no active efforts to breed or maintain Eastern Sand Darter under human care in Canada; therefore, individuals from a wild source population(s) are needed to implement reintroduction at this time. Populations outside of the southwestern Ontario DU were not considered as potential sources to avoid genetic or demographic effects that may result from translocating individuals from outside the region. The Grand River and Thames River populations were considered as the candidate source populations for an experimental Eastern Sand Darter reintroduction, as these populations are the most well-studied and have more secure population status relative to other extant populations (COSEWIC 2023, DFO 2023).

## **Step 2.1 – Assess the probability that successful reintroduction improves survival or recovery of Eastern Sand Darter and the ability to achieve a successful reintroduction**

Establishing a population can improve the survival or recovery of a species by reducing the likelihood of species extinction given stochastic and (or) catastrophic events among populations. Basic simulations illustrate how the number of populations, and the persistence of those populations, affects long-term species viability. There are currently eight populations of Eastern Sand Darter in southwestern Ontario; two populations in ‘Good’ condition (Grand and Thames rivers), four populations in ‘Poor’ condition (Lake St. Clair, Sydenham River, Long Point Bay, Big Creek), and two populations with an ‘Unknown’ status (Lake Erie Western Basin, Rondeau Bay; DFO 2011). Simulations were used to illustrate how the addition of a reintroduced population could reduce the extinction probability of the southwestern Ontario DU and therefore improve the survival or recovery of the species. The simulations suggested that a reintroduced (ninth) population in ‘Good’ condition would reduce median extinction probability by 97.20% while a reintroduced population in ‘Poor’ condition would reduce median extinction probability by 63.09%. Note, however, that the probability of persistence for each population is generally uncertain; as such, the results of the simulations were interpreted qualitatively rather than as true extinction probabilities.

### **Estimating population abundance**

Indices of abundance were generated for Eastern Sand Darter in the Grand and Thames rivers using *N*-mixture models and estimates of suitable habitat availability. Models were developed independently for the two river systems due to differences in available data.

#### *Grand River Indices of Abundance*

Data for modelling Eastern Sand Darter abundance in the Grand River were collected in 2022 (Gáspárdy et al. 2025) in the Brantford and Caledonia sections of the river (Figure 3). One hundred sites were sampled, with 54 in the Brantford section and 46 in the Caledonia section. In total, 610 Eastern Sand Darter were captured with 0–174 individuals detected per site. Modeling to estimate density included 96 of the 100 sampled sites, with four sites excluded due to missing covariate data. Model-estimated mean site density was higher in the Brantford section (0.0754 fish/m<sup>2</sup>; 95% CI: 0.0359–0.1811) than the Caledonia section (0.0191 fish/m<sup>2</sup>; 95% CI: 0.0079–0.0535). Note that density estimates are based on sampling in wadable habitats and may not represent density in non-wadable habitats. Eastern Sand Darter density was generally higher at sites that had substrate with a high percent of sand or gravel and with low turbidity.

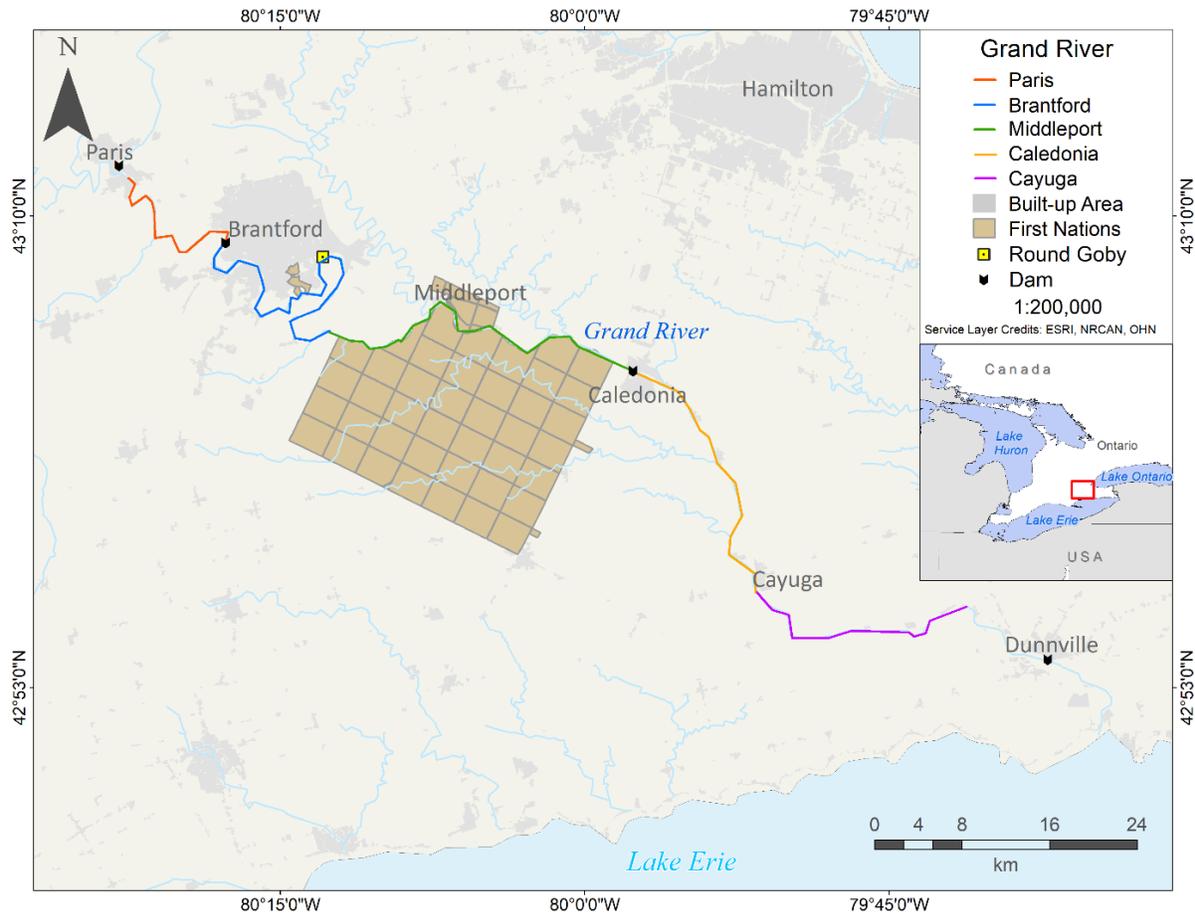


Figure 3. Map of areas containing critical habitat in the Grand River, divided into five study segments (colours). Farthest upstream detection record (2022) of Round Goby (*Neogobius melanostomus*) indicated with the yellow square.

A resampling with replacement statistical procedure was used to estimate the abundance of age-1+ Eastern Sand Darter in the Grand River based on an assumed amount of occupied suitable habitat (i.e., substrate with at least 50% sand and fine gravel composition) that was considered wadable or non-wadable, both of which varied by river section. Assuming equivalent densities between wadable and non-wadable habitats, median age-1+ population abundance in the Brantford section ranged between 9,438–26,051 in wadable habitat (Figure 4) and 22,021–60,786 in non-wadable habitat (Figure 5). Abundance estimates for sections downstream of the Brantford section were significantly lower given the lower density estimates and lower proportion of suitable substrate; median abundance ranged between 356–4,357 age-1+ individuals in wadable areas of the Middleport, Caledonia, and Cayuga sections, and 1,416–11,395 in non-wadable habitats. Across sections and suitable habitat scenarios, median population abundance was estimated to be between 41,965–119,159 age-1+ individuals in the Grand River (Brantford: 31,459–86,837; Middleport: 4,116–12,661; Caledonia: 2,832–8,714; Cayuga: 3,558–10,947). Figure 4 and Figure 5 demonstrate how the proportion of total habitat that is suitable for Eastern Sand Darter in wadable and non-wadable habitats, respectively, influenced population abundance estimates.

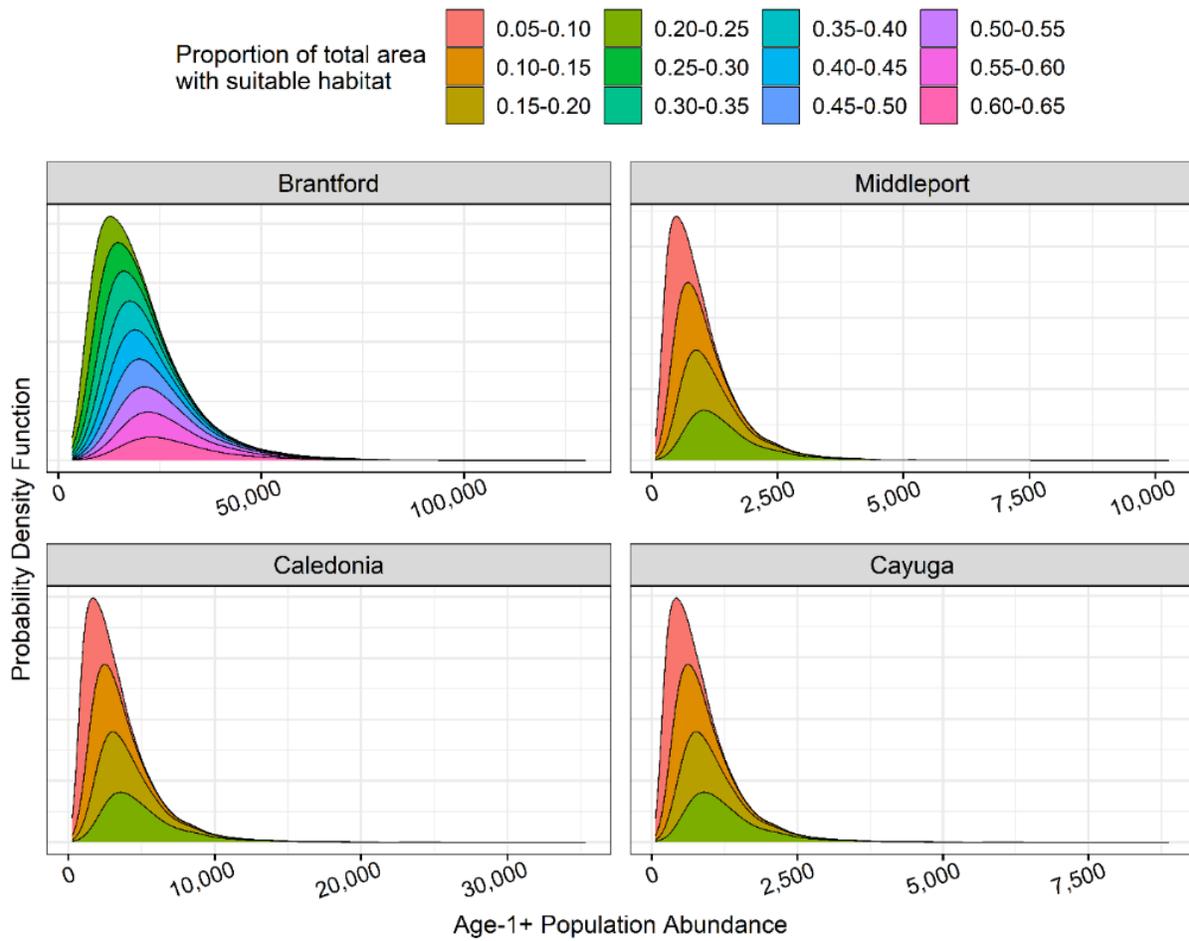


Figure 4. Indices of age-1+ Eastern Sand Darter population abundance in wadable sections of the Brantford, Middleport, Caledonia, and Cayuga sections of the Grand River based on the proportion of total habitat with suitable substrate (colours) and model-generated densities.

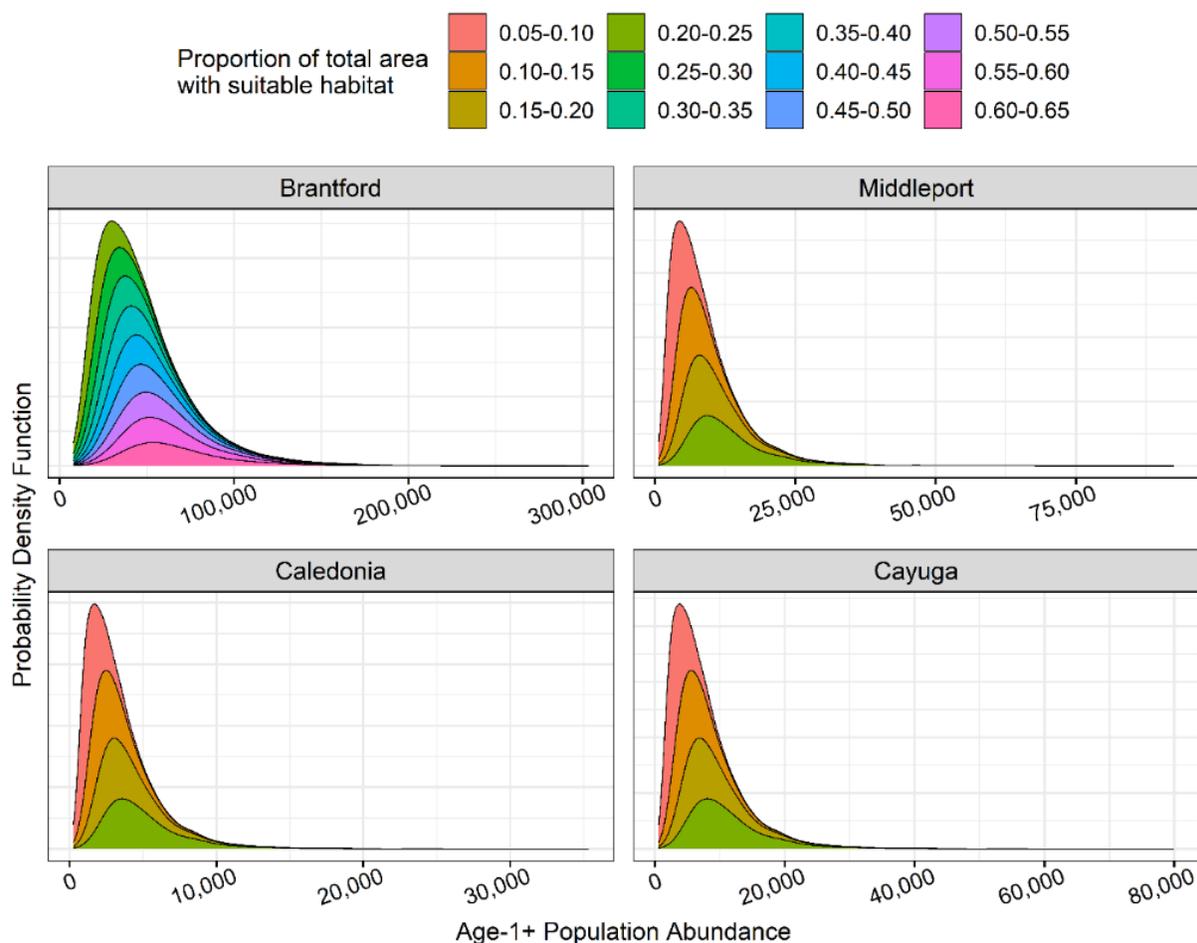


Figure 5. Indices of age-1+ Eastern Sand Darter population abundance across in non-wadable sections of the Brantford, Middleport, Caledonia, and Cayuga sections of the Grand River based on the proportion of total habitat with suitable substrate (colours) and model-generated densities.

#### Thames River Indices of Abundance

Recent field data were unavailable for estimating age-1+ abundance of Eastern Sand Darter in the Thames River; therefore, data collected in 2006 (Dextrase 2013) were used to build  $N$ -mixture models to estimate abundance. Mean site density for Eastern Sand Darter in the Thames River was estimated to be 0.040 fish/m<sup>2</sup> (95% CI: 0.026–0.066). As with the Grand River, mean site density increased with water clarity and when sites had substrate with higher proportions of sand and fine gravel. Site-level density estimates were made for the Thames River based on average site characteristics, which differed from the Grand River.

Estimates of age-1+ population abundance were made for wadable and non-wadable areas across the entire area containing Eastern Sand Darter critical habitat in the Thames River (approximately 5,890,400 m<sup>2</sup>). A statistical resampling with replacement approach was again used to estimate the abundance of Eastern Sand Darter based on the potential area of suitable habitat (i.e., substrate with at least 50% sand and fine gravel composition). Moreover, approximately 50% of the total habitat was considered to be wadable based on acoustic doppler

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current profiler data collected in the Thames River between Komoka and Kent Bridge (Illes et al. 2025). Age-1+ abundance estimates in the Thames River were equivalent between wadable and non-wadable habitats given that approximately 50% of the habitat was considered wadable. Median abundance across wadable and non-wadable habitats combined ranged between 17,030–108,744 age-1+ individuals (Figure 6).

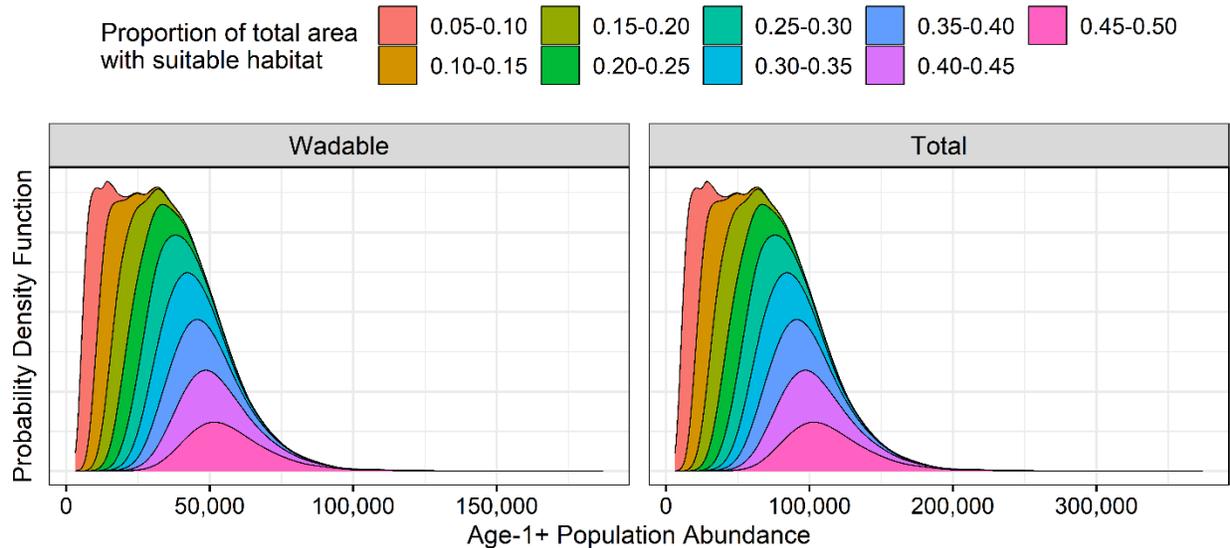


Figure 6. Indices of age-1+ Eastern Sand Darter population abundance in wadable sections (equivalent for non-wadable), and overall (Total), of the Thames River based on the proportion of total habitat with suitable substrate (colours) and model-generated densities.

### Potential abundance in the Ausable River and Big Otter Creek

The potential abundance of Eastern Sand Darter that could theoretically be achieved in candidate recipient locations was estimated based on habitat availability and assuming similar densities to the source populations. Mean potential population abundance in the Ausable River was 30,488 age-1+ individuals (median = 22,499; Figure 7) across the full range of habitat values. At the lowest level of suitable habitat (i.e., 5–10% of total habitat), median potential abundance was 6,766 age-1+ individuals while median potential abundance was 43,221 individuals when suitable habitat was between 45–50% of the total habitat (Figure 7). These estimates assume that 5–50% of total habitat would be suitable and potentially occupied, and that density of Eastern Sand Darter would be similar to the Grand River (Brantford and Caledonia sections) and Thames River.

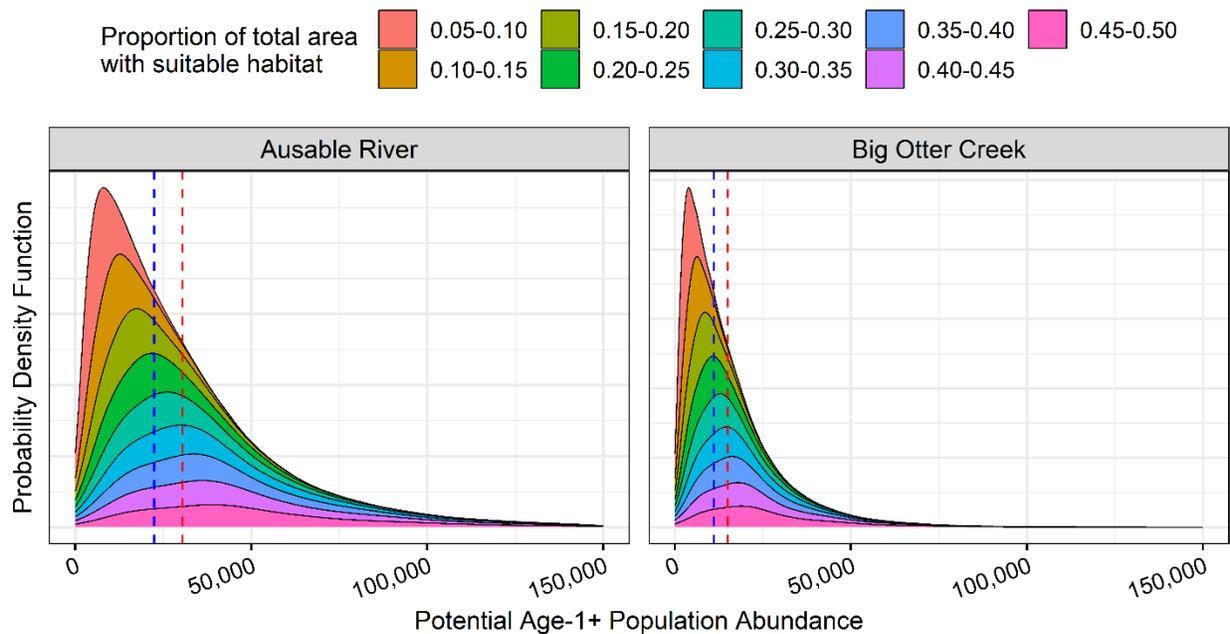


Figure 7. Kernel density plot of potential abundance estimates for a reintroduced population of Eastern Sand Darter in the Ausable River and Big Otter Creek based on the estimated area of suitable habitat (colours) and model-generated density estimates from the Brantford and Caledonia sections of the Grand River, and the Thames River. Dashed lines indicate median (blue) and mean (red) potential population abundances.

Potentially suitable habitat for Eastern Sand Darter in Big Otter Creek is expected to occur upstream of Vienna to Otterville, Ontario. Mean potential abundance in Big Otter Creek was estimated as 14,994 (median = 11,027) across the full range of habitat values (Figure 7). At the lowest level of suitable habitat (i.e., 5–10% of total habitat), median potential abundance was 3,341 individuals while median potential abundance was 21,289 when suitable habitat was between 45–50% of the total habitat (Figure 7).

#### Modelling the trade-off between removals from the Grand and (or) Thames rivers and the probability of establishment in recipient locations

Methods for evaluating the trade-off between removals from source populations and the probability of successful re-establishment were similar to those presented in Lamothe et al. (2021). The life cycle of Eastern Sand Darter was modelled using a female-only, density-dependent, age-structured, birth-pulse, pre-breeding matrix model with annual projection intervals (Caswell 2001). Minimum viable population ( $MVP_{99\%}$ ) was estimated for Eastern Sand Darter, which describes the absolute minimum population size of age-1+ individuals that has a 99% probability of remaining extant over 100 years despite the continuous effects of stochasticity and catastrophic events (Shaffer 1981). The mean and variance of  $MVP_{99\%}$  were used as criteria for evaluating the risk to source populations when removing individuals, and the successful establishment of Eastern Sand Darter in the recipient location. Mean estimated  $MVP_{99\%}$  for Eastern Sand Darter was 16,651 age-1+ females (95% CI: 3,098–51,229), or approximately 33,302 age-1+ individuals of both sexes.

*Effects of removals on source populations*

A population viability analysis was used to quantify the impact of removing individuals from a source population. Annual removals were performed for 1 to 10 years, with 0 to 500 age-1+ females removed annually. Significant harm to the source populations was considered to occur if the source population declined below mean  $MVP_{99\%}$  (16,651 age-1+ females) at any point during the simulation, indicating a chance that the population could be extirpated over the next 100 years. Logistic regression was used to identify influential covariates on the probability of removal impacts. The two most influential variables on the probability of removal impacts were source population size and the probability of catastrophe. As source population abundance increased, the probability of removal impacts decreased (Figure 8) whereas the probability of removal impacts increased with increased rates of catastrophes.

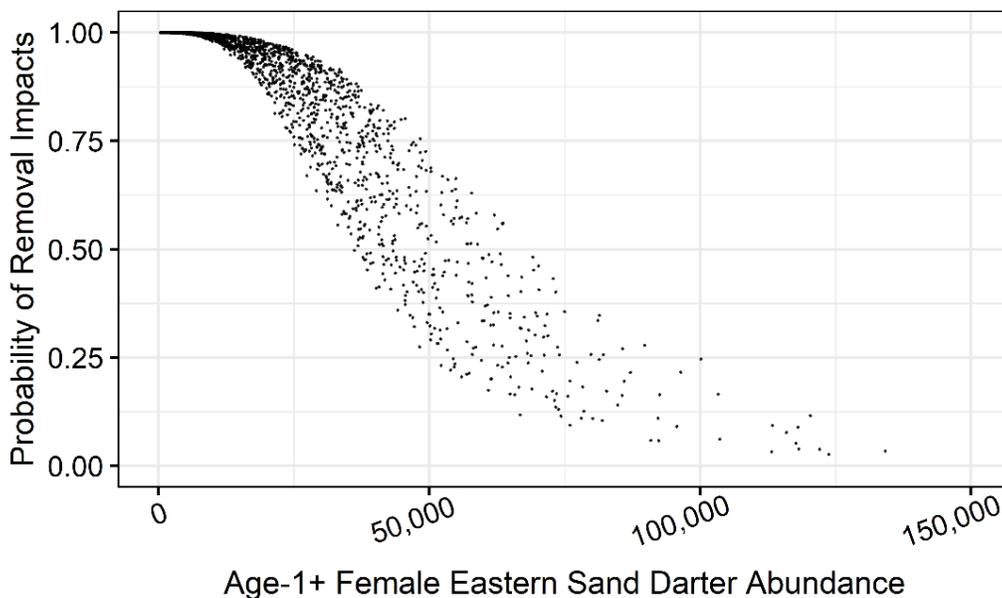


Figure 8. Probability of reducing abundance below the mean minimum viable population ( $MVP_{99\%}$ ) size (i.e., probability of removal impacts) as a function of age-1+ female Eastern Sand Darter abundance.

**Step 2.2 – Information in support of estimating the probability of establishing a self-sustaining population of Eastern Sand Darter in the recipient locations**

Many factors can influence reintroduction success. The questions listed in Table 1 were developed to understand how certain factors may influence the probability of successful reintroduction for Eastern Sand Darter in southwestern Ontario (i.e., ecological feasibility). Following these questions, a brief summary of the best available information is presented (see Lamothe et al. 2023 for further detail). Participants in the CSAS meeting were asked to answer these questions during the meeting based on the available evidence, while simultaneously providing a measure of uncertainty based on their interpretation and evaluation of the question (Described in Step 4 – Compile and weigh the scientific evidence).

**Grand and Thames river population abundance and the probability of harm resulting from removals (Questions 1, 2, 5, 6)**

Population abundance was estimated for Eastern Sand Darter by extrapolating model-estimated densities across areas of potentially suitable habitat in the Grand and Thames rivers. Significant uncertainty in abundance estimates was caused by a limited understanding of the true amount of suitable habitat in both rivers and whether density estimates of wadable habitat could be extrapolated to non-wadable habitat. Age-1+ population density and abundance estimates were higher in the Grand River than the Thames River. Model-generated population abundance estimates often fell below mean  $MVP_{99\%}$  during the removal simulations for both river populations, but the magnitude of removals had a minimal effect on the probability of falling below mean  $MVP_{99\%}$ . Due to the lower estimated abundance in the Thames River, the probability of harm was higher for the Thames River population than the Grand River. These model results involved high uncertainty, including uncertainty from initial model-generated density estimates, extrapolation of abundance based on an uncertain availability of suitable habitat, and population models used to simulate removals and introductions.

**Influence of population-specific life-history characteristics on establishment of a self-sustaining population (Questions 3, 7)**

Most life-history information for Eastern Sand Darter was obtained from the Thames River, with limited information from the Grand River. Length data for both populations indicate that greater adult lengths are obtained in the Grand River than in the Thames River. Variation in life-history parameters was incorporated into the population models used to evaluate the effects of removals and additions. Consequently, the results from the removal and addition models had significant uncertainty. Determining the true values of these parameters would improve accuracy and precision of  $MVP_{99\%}$  estimates and subsequently improve the ability to quantify the effects of removals on source populations.

**Influence of population genetic diversity, variation, and (or) adaptation in the Grand River on the establishment and persistence of a reintroduced population (Questions 4, 8)**

A source population selection framework was previously developed that considered genetic ancestry and environmental requirements (Meffe 1995, Reisenbichler et al. 2003, Houde et al. 2015). Similar to other fishes of the Great Lakes basin, genetic structure of Eastern Sand Darter is explained by post-glacial dispersal following the Wisconsinan period (Mandrak and Crossman 1992). This suggests that populations in relatively close geographic proximity are most closely related. Population genetics studies have been performed for Eastern Sand Darter (Ginson et al. 2015, Walter et al. 2022). Microsatellite DNA locus allelic richness and observed heterozygosity in the Grand and Thames river populations were comparable to other populations across the species' North American range. Low gene flow was observed between populations, but low levels of within-river genetic structure in the Grand and Thames rivers suggested movement of individuals between habitat patches (Ginson et al. 2015).

Table 1. Population, habitat (abiotic and biotic), and threat considerations for evaluating the ability to reintroduce Eastern Sand Darter in the Ausable River or Big Otter Creek, Ontario.

No.	Category	Focal location	Question
1a	Population	Grand River	What is the probability that population abundance is greater than 25,000 age-1+ individuals?
1b	Population	Grand River	What is the probability that population abundance is greater than 50,000 age-1+ individuals?
1c	Population	Grand River	What is the probability that population abundance is greater than 100,000 age-1+ individuals?
2a	Population	Grand River	What is the probability that the population would allow the removal of 250 age-1+ individuals per year for up to 10 years without reducing the population below MVP <sub>99%</sub> during, and at least three generations after, removal efforts?
2b	Population	Grand River	What is the probability that the population would allow the removal of 500 age-1+ individuals per year for up to 10 years without reducing the population below MVP <sub>99%</sub> during, and at least three generations after, removal efforts?
2c	Population	Grand River	What is the probability that the population would allow the removal of 1,000 age-1+ individuals per year for up to 10 years without reducing the population below MVP <sub>99%</sub> during, and at least three generations after, removal efforts?
3	Population	Grand River	What is the probability that population-specific life-history characteristics (e.g., age-at-maturity, fecundity, survival, growth, sex ratio) would prevent the establishment of a self-sustaining population?
4a	Population	Grand River	What is the probability that genetic diversity, variation, and (or) adaptation would prevent establishment of a reintroduced population in the Ausable River?
4b	Population	Grand River	What is the probability that genetic diversity, variation, and (or) adaptation would prevent a reintroduced population from reaching MVP <sub>99%</sub> in the Ausable River?
4c	Population	Grand River	What is the probability that genetic diversity, variation, and (or) adaptation would prevent establishment of a reintroduced population in Big Otter Creek?
4d	Population	Grand River	What is the probability that genetic diversity, variation, and (or) adaptation would prevent a reintroduced population from reaching MVP <sub>99%</sub> in Big Otter Creek?

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No.	Category	Focal location	Question
5a	Population	Thames River	What is the probability that population abundance is greater than 25,000 age-1+ individuals?
5b	Population	Thames River	What is the probability that population abundance is greater than 50,000 age-1+ individuals?
5c	Population	Thames River	What is the probability that population abundance is greater than 100,000 age-1+ individuals?
6a	Population	Thames River	What is the probability that the population would allow the removal of 250 age-1+ individuals per year for up to 10 years without reducing the population below MVP <sub>99%</sub> during, and at least three generations after, removal efforts?
6b	Population	Thames River	What is the probability that the population would allow the removal of 500 age-1+ individuals per year for up to 10 years without reducing the population below MVP <sub>99%</sub> during, and at least three generations after, removal efforts?
6c	Population	Thames River	What is the probability that the population would allow the removal of 1,000 age-1+ individuals per year for up to 10 years without reducing the population below MVP <sub>99%</sub> during, and at least three generations after, removal efforts?
7	Population	Thames River	What is the probability that population-specific life-history characteristics (e.g., age-at-maturity, fecundity, survival, growth, sex ratio) would prevent the establishment of a self-sustaining population?
8a	Population	Thames River	What is the probability that genetic diversity, variation, and (or) adaptation would prevent establishment of a reintroduced population in the Ausable River?
8b	Population	Thames River	What is the probability that genetic diversity, variation, and (or) adaptation would prevent a reintroduced population from reaching MVP <sub>99%</sub> in the Ausable River?
8c	Population	Thames River	What is the probability that genetic diversity, variation, and (or) adaptation would prevent establishment of a reintroduced population in Big Otter Creek?
8d	Population	Thames River	What is the probability that genetic diversity, variation, and (or) adaptation would prevent a reintroduced population from reaching MVP <sub>99%</sub> in Big Otter Creek?
9a	Habitat (abiotic)	Ausable River	What is the probability that suitable abiotic conditions are available for Eastern Sand Darter?
9b	Habitat (abiotic)	Ausable River	What is the probability that suitable abiotic conditions are available in sufficient quantity to support 5,000 Eastern Sand Darter individuals?

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No.	Category	Focal location	Question
9c	Habitat (abiotic)	Ausable River	What is the probability that suitable abiotic conditions are available in sufficient quantity to support a population abundance equal to MVP <sub>99%</sub> ?
10	Habitat (abiotic)	Ausable River	What is the probability that there is sufficient connectivity between habitats to support all life-stages of a reintroduced population?
11a	Habitat (abiotic)	Big Otter Creek	What is the probability that suitable abiotic conditions are available for Eastern Sand Darter?
11b	Habitat (abiotic)	Big Otter Creek	What is the probability that suitable abiotic conditions are available in sufficient quantity to support 5,000 Eastern Sand Darter individuals?
11c	Habitat (abiotic)	Big Otter Creek	What is the probability that suitable abiotic conditions are available in sufficient quantity to support a population abundance equal to MVP <sub>99%</sub> ?
12	Habitat (abiotic)	Big Otter Creek	What is the probability that there is sufficient connectivity between habitats to support all life-stages of a reintroduced population?
13a	Habitat (biotic)	Ausable River	What is the probability that suitable food resources for Eastern Sand Darter are available?
13b	Habitat (biotic)	Ausable River	What is the probability that suitable food resources for Eastern Sand Darter are available in sufficient quantity to support 5,000 Eastern Sand Darter individuals?
13c	Habitat (biotic)	Ausable River	What is the probability that suitable food resources for Eastern Sand Darter are available in sufficient quantity to support a population abundance equal to MVP <sub>99%</sub> ?
14a	Habitat (biotic)	Big Otter Creek	What is the probability that suitable food resources for Eastern Sand Darter are available?
14b	Habitat (biotic)	Big Otter Creek	What is the probability that suitable food resources for Eastern Sand Darter are available in sufficient quantity to support 5,000 Eastern Sand Darter individuals?
14c	Habitat (biotic)	Big Otter Creek	What is the probability that suitable food resources for Eastern Sand Darter are available in sufficient quantity to support a population abundance equal to MVP <sub>99%</sub> ?
15a	Habitat (biotic)	Ausable River	What is the probability that competition would prevent the establishment of Eastern Sand Darter during 10 years of reintroduction efforts?
15b	Habitat (biotic)	Ausable River	What is the probability that predation would prevent the establishment of Eastern Sand Darter during 10 years of reintroduction efforts?

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No.	Category	Focal location	Question
16a	Habitat (biotic)	Big Otter Creek	What is the probability that competition would prevent the establishment of Eastern Sand Darter during 10 years of reintroduction efforts?
16b	Habitat (biotic)	Big Otter Creek	What is the probability that predation would prevent the establishment of Eastern Sand Darter during 10 years of reintroduction efforts?
17	Threats	Ausable River	What is the probability that agricultural activities would prevent the establishment of an Eastern Sand Darter population across 10 years of reintroduction efforts?
18	Threats	Ausable River	What is the probability that Round Goby would prevent the establishment of an Eastern Sand Darter population across 10 years of reintroduction efforts?
19	Threats	Ausable River	What is the probability that pathogens or parasites would prevent the establishment of an Eastern Sand Darter population across 10 years of reintroduction efforts?
20	Threats	Big Otter Creek	What is the probability that agricultural activities would prevent the establishment of an Eastern Sand Darter population across 10 years of reintroduction efforts?
21	Threats	Big Otter Creek	What is the probability that Round Goby would prevent the establishment of an Eastern Sand Darter population across 10 years of reintroduction efforts?
22	Threats	Big Otter Creek	What is the probability that pathogens or parasites would prevent the establishment of an Eastern Sand Darter population across 10 years of reintroduction efforts?

### **Eastern Sand Darter habitat requirements**

Eastern Sand Darter is a habitat specialist that requires sand and fine gravel substrate throughout its life-history. Research on southwestern Ontario populations of Eastern Sand Darter has demonstrated a strong positive association between the amount of sand and fine gravel substrate at a site and the presence, abundance, and size of Eastern Sand Darter (Drake et al. 2008, Dextrase 2013, 2014). As well, experimental research has demonstrated that Eastern Sand Darter has an ability to tolerate relatively high temperatures and low levels of dissolved oxygen (Firth et al. 2021a, 2023).

The habitat area required to sustain an Eastern Sand Darter population is generally unknown. The minimum area for population viability (*MAPV*) was calculated, which describes the amount of suitable habitat required for supporting a population at *MVP*<sub>99%</sub> (Vélez-Espino et al. 2010). Median *MAPV* for Eastern Sand Darter was approximately 339,201 m<sup>2</sup> based on density estimates from the Brantford section of the Grand River, 1,324,601 m<sup>2</sup> based on density estimates from the Caledonia section of the Grand River, and 636,486 m<sup>2</sup> based on density estimates from the Thames River. Total habitat was estimated to be 2,301,090 m<sup>2</sup> in the Ausable River and 1,134,382 m<sup>2</sup> in Big Otter Creek.

Habitat matching was performed for several habitat characteristics hypothesized to be important for Eastern Sand Darter in southwestern Ontario. Comparisons of the mean, variance, and range of habitat characteristics were made between measurements from potential recipient river systems and sites where Eastern Sand Darter was captured in the Grand and Thames rivers. Data used for habitat matching spanned from 2002–2022, where data were collected with different objectives, methodologies, and sample sizes across rivers.

Generally, the percent sand cover in the Ausable River and Big Otter Creek was lower than the potential source rivers at sites where Eastern Sand Darter has been detected. However, targeted habitat sampling performed in Big Otter Creek revealed a mean percent composition of sand and fine gravel of 89.8% and 95.3% at six sites within and six sites beyond historical detections of Eastern Sand Darter, respectively (Barnucz et al. 2022). These mean values exceed the percent composition of sand and fine gravel at a site where Eastern Sand Darter has been captured consistently in the Grand River over the past decade (Cockshutt Bridge, Brantford).

Targeted dissolved oxygen (DO; mg/L) measurements were taken in Big Otter Creek to allow comparisons with sites occupied by Eastern Sand Darter in the Grand River (Barnucz et al. 2022). Specifically, differences in DO at the substrate surface and within the substrate (depth = 1 cm) were compared between river systems (Grand River, Big Otter Creek historical sites, Big Otter Creek targeted sites). Due to seasonal differences between surveys, the difference in DO between the surface and 1 cm into the substrate was calculated rather than directly comparing DO measurements. Differences in surface and subsurface dissolved oxygen concentrations were significantly different between collection locations; targeted sites in Big Otter Creek had a greater difference in DO between surface and subsurface dissolved oxygen (9.69 mg/L ± 2.76 SD) compared to historical sites in Big Otter Creek (7.44 ± 3.59) and the Grand River (7.44 ± 2.56). This suggests that there is less DO available 1 cm into the substrate at targeted sites in Big Otter Creek, but historical sites are similar to those in the Grand River.

**Connectivity in the Ausable River and Big Otter Creek for supporting all life-stages  
(Questions 10, 12)**

Identifying the degree of connectivity to support a population requires an understanding of the life-history of the species, and direction and magnitude of individual movements within and among self-sustaining populations. Minns (1995) developed an allometric relationship between home range size and body length (mm) for riverine fishes. Assuming a maximum length of 56 mm, home range size for riverine populations of Eastern Sand Darter is 42 m<sup>2</sup>.

There are no major barriers (dams) on the main stem of the Ausable River downstream of Exeter, Ontario. There are two primary flow regulation structures in the Big Otter Creek watershed. Norwich Dam is located on a tributary to Big Otter Creek in the headwaters, upstream of potential Eastern Sand Darter habitat. The second dam is located on the main stem at Otterville, Ontario, marking the upstream boundary of potential Eastern Sand Darter habitat. As well, small dams exist on virtually all tributaries of Big Otter Creek to store water for irrigation (Loomer 2011). Overall, there are fewer barriers in the mainstem of the Ausable River and Big Otter Creek than the Grand River.

**Availability of food resources in the Ausable River (Questions 13, 14)**

Sufficient food resources are needed to support a reintroduced population of Eastern Sand Darter in the recipient location. The diet of Eastern Sand Darter has been described as consisting of larval Chironomidae, Ostracoda, Cladocera, Ephemeroptera, and Oligochaeta based on stomach contents (Burbank et al. 2019).

A benthic macroinvertebrate sampling data set was provided by the Ausable Bayfield Conservation Authority for the Ausable River. Across the three surveyed sites, 17 orders, 37 families, and at least 21 genera of benthic macroinvertebrates were identified. Although only one genus from the Chironomidae family was identified (*Cricotopus* sp.), chironomids made up 18.8% of the captures across the three sites, with the most upstream site having the greatest relative density. Six Ephemeroptera families were detected, composed of at least five genera.

In October 2022, benthic macroinvertebrates were sampled from six sites in Big Otter Creek. Of the six sites, four were historically occupied locations whereas the other two were considered potentially suitable for Eastern Sand Darter based on substrate characteristics (Barnucz et al. 2022). Across the six surveyed sites, 12 orders, 20 families, and 32 genera of benthic macroinvertebrates were identified. *Hesperocorixa vulgaris* (Hemiptera) was identified as the most abundant taxa ( $n = 323$  individuals), while *Choronomus* sp. (Chironomidae) was present at the most sites ( $n = 5$  sites). Four and five genera in the Chironomidae family and Ephemeroptera group were detected across the six sites, respectively, which were previously identified as major components of Eastern Sand Darter diet (Burbank et al. 2019).

**Influence of co-occurring species on reintroduction (Questions 15, 16)**

There is no research that suggests Eastern Sand Darter has an obligate, facultative, or parasitic species dependency that would limit its ability to be successfully reintroduced. No studies have quantified direct species interactions with Eastern Sand Darter, but species co-occurrence patterns have been investigated (Lamothe et al. 2019a,b). Eastern Sand Darter has been identified as positively associated with benthic species such as Bluntnose Minnow (*Pimephales notatus*) and Northern Hogsucker (*Hypentelium nigricans*), and negatively associated with Emerald Shiner (*Notropis atherinoides*) and Rock Bass (*Ambloplites rupestris*; Lamothe et al. 2019a). The mechanisms behind these relationships are unknown, but are likely related to species-habitat preferences rather than direct interactions.

Species lists were generated to compare fish communities between potential source locations (areas containing Eastern Sand Darter critical habitat) and recipient locations (Ausable River: upstream of 'The Cut'; Big Otter Creek: upstream of Vienna to Otterville); however, the data used to generate these lists differed significantly by frequency of sampling, gear type, and the spatial extent of sampling, and therefore caution was warranted around interpretation of community differences. Based on these data, the Grand River has supported at least 61 species of fishes over the period of sampling (2002–2022), Big Otter Creek has supported at least 33 species, and the middle and upstream sections of the Ausable River have supported at least 66 species. In total, 21 predators and 56 competitors of Eastern Sand Darter were identified across the potential source and recipient systems. The Ausable River and Thames River both support at least 17 presumed predators of Eastern Sand Darter, while the Grand River supports at least 12, and Big Otter Creek supports at least seven. The Thames River, Ausable River, Grand River, and Big Otter Creek support at least 44, 42, 36, and 24 presumed competitors, respectively.

#### **Agricultural activities (Questions 17, 20)**

Increased turbidity and sediment loading, along with the introduction of contaminants, nutrients, and toxic substrates, are common consequences of agricultural practices on freshwater ecosystems. Moreover, turbidity and sediment loading have been identified as the leading cause of habitat loss for Eastern Sand Darter and a likely cause of extirpation (Holm and Mandrak 1996, COSEWIC 2009, DFO 2012). Rivers that support Eastern Sand Darter populations in southwestern Ontario are all located within watersheds where the primary form of land-use is agriculture. Similarly, the Ausable River watershed and the Big Otter Creek sub-watershed are dominated by agriculture.

#### **Round Goby (Questions 18, 21)**

Round Goby is identified as a threat to the persistence of Eastern Sand Darter in Ontario due to presumed competitive interactions and diet overlap (DFO 2012, 2018, Firth et al. 2021b). In 2017, Round Goby was detected in the Ausable River as far upstream as Elginfield Rd. in Sylvan, Ontario, with no detections of Round Goby at sites sampled further upstream in Arkona, Ontario. However, sampling downstream of Ailsa Craig has not occurred since 2017.

Results from targeted sampling for Eastern Sand Darter across 52 sites in Big Otter Creek in 2018 indicated that Round Goby was among the most abundant and frequently encountered species in the river. Round Goby was captured in the main branch of Big Otter Creek, as far upstream as Tillsonburg, Ontario (Barnucz et al. 2020, McAllister et al. 2022); therefore, the distribution of Round Goby in Big Otter Creek overlaps with all historical records of Eastern Sand Darter (Barnucz et al. 2020).

#### **Disease (Questions 19, 22)**

Diseases or parasites at the recipient location could prevent the establishment of reintroduced individuals through mortality or reduced fitness following release, whether the disease was already present in the recipient location or spread during the process of moving individuals between locations (Viggers et al. 1993, Sainsbury et al. 2012). There is limited knowledge about the impacts of disease or parasites on Eastern Sand Darter and for most fishes that it co-occurs with.

**Step 3 – Information in support of estimating the probability of unintended consequences from Eastern Sand Darter reintroduction**

The third step in the decision support framework is to identify and assess the probability of unintended negative consequences of reintroduction on the source and recipient populations and ecosystems (Figure 2). Similar to Step 2, the goal of Step 3 is to assess the probability of unintended consequences occurring due to the removal and (or) establishment of individuals in the source and recipient locations (Table 2). Below, questions are presented that relate to the probability of unintended consequences occurring as a result of reintroduction efforts, with answers provided based on best-available information. Similar to the previous section, these questions were developed for experts to answer during the meeting based on the available evidence, and provide a measure of uncertainty based on their interpretation and evaluation of the question.

*Table 2. Ecological risk considerations for the focal taxa and other ecosystem components in source and recipient habitats of proposed reintroductions.*

No.	Question
23a	What is the probability of an increased rate of inbreeding depression in the source population during, or three generations following, the removal of 250 age-1+ individuals per year for up to 10 years?
23b	What is the probability of an increased rate of inbreeding depression in the source population during, or three generations following, the removal of 500 age-1+ individuals per year for up to 10 years?
23c	What is the probability of an increased rate of inbreeding depression in the source population during, or three generations following, the removal of 1,000 age-1+ individuals per year for up to 10 years?
24a	What is the probability of inbreeding depression in the reintroduced population during, or three generations following, the addition of 250 age-1+ individuals per year for up to 10 years?
24b	What is the probability of inbreeding depression in the reintroduced population during, or three generations following, the addition of 500 age-1+ individuals per year for up to 10 years?
24c	What is the probability of inbreeding depression in the reintroduced population during, or three generations following, the addition of 1,000 age-1+ individuals per year for up to 10 years?
25a	What is the probability of a founder effect occurring in the recipient location given the reintroduction of 250 age-1+ individuals per year for up to 10 years?
25b	What is the probability of a founder effect occurring in the recipient location given the reintroduction of 500 age-1+ individuals per year for up to 10 years?
25c	What is the probability of a founder effect occurring in the recipient location given the reintroduction of 1,000 age-1+ individuals per year for up to 10 years?
26	What is the probability of outbreeding depression in the recipient location if the Grand and Thames river populations were both used to source a reintroduction?
27	What is the probability of interspecific hybridization in the recipient location?
28a	What is the probability that reintroduction would introduce novel pathogens or parasites to the recipient location?

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No.	Question
28b	What is the probability that introduced novel pathogens or parasites would cause significant harm to the recipient freshwater community?
29	What is the probability of transformative changes occurring in the source location within and outside of areas of removals as a result of removing 1,000 individuals per year for 10 years?
30	What is the probability of transformative changes occurring in the recipient location within and outside of areas of additions as a result of reintroducing 1,000 individuals per year for 10 years?

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**Inbreeding depression in the source and recipient population (Questions 23, 24)**

Inbreeding depression describes the relative reduction in fitness of offspring resulting from the mating of closely related individuals compared to those of randomly mated individuals (Hedrick and Kalinowski 2000). Removals from populations of smaller size and site-level densities are more likely to experience inbreeding depression than large populations with high site-level abundance. Similarly, introductions of relatively few individuals at a site could result in inbreeding depression in the recipient system. Assuming reintroduction was to occur with hundreds of individuals across multiple years with individuals sampled from across the within-river range of the species, inbreeding depression in the source and recipient locations is likely to be of minimal concern.

**Founder effect in the recipient location (Question 25)**

The founder effect describes the loss in genetic variation incurred as a result of establishing a population with a small number of individuals unrepresentative of the species pool (Jamieson 2011). This effect is commonly described for invasive species and captive breeding programs and can be of concern for reintroduction efforts. Multiple introductions of randomly selected, unrelated individuals with sufficient genetic diversity from the source to recipient location can reduce the likelihood of experiencing founder effects (Le Gouar et al. 2008, Alcaide et al. 2010). This can be achieved by sourcing individuals across its entire within-river range, which would also reduce the impact of harm at the site level.

**Outbreeding depression as a result of mixing Grand and Thames river populations (Question 26)**

Outbreeding depression is defined as a reduction in fitness caused by the crossing of genetically distinct populations. In the scenario where a recipient population has established, outbreeding depression could be observed as reduced fertility, or fitness-related surrogates, of individuals relative to either of the source populations. In turn, reduced fitness of the reintroduced population would make it more vulnerable to collapse in the face of natural and anthropogenic disturbances. If a single source population is considered for use, outbreeding depression is of no concern.

**Interspecific hybridization in the recipient location (Question 27)**

Interspecific hybridization can lead to a variety of outcomes for reintroduction efforts, none of which will benefit the conservation of Eastern Sand Darter. Eastern Sand Darter comes from a monophyletic group indicating that it has a single ancestor that differs from most other darter species (Near et al. 2011). There are no other species of the genus *Ammocrypta* in Canada. There are no records of hybridization between Eastern Sand Darter and co-occurring species

despite co-occurring with several darter species of two genera (*Etheostoma* and *Percina* species) in the Grand and Thames rivers.

**Introducing disease to the recipient location (Question 28)**

Translocating individuals from one location to another poses risks of moving pathogens and parasites that may harm individuals in the recipient population. Most areas in source and recipient locations are subject to movement of migratory species between watersheds, and therefore it's expected that disease prevalence is similar between source and recipient locations. However, no studies have been performed to support this hypothesis and there is limited knowledge about the impacts of pathogens and parasites on Eastern Sand Darter and co-occurring fishes.

**Transformative changes in source locations (Question 29)**

The removal of fishes from an ecosystem can lead to significant changes in fish community dynamics by making resources available for other individuals or species to exploit. The probability of transformative change is directly linked to the number and frequency of removals. If too many Eastern Sand Darter are removed from a population, there is a risk that other species may capitalize on the available resources. In such a scenario, constraints on the growth of other species may be reduced and result in a new hierarchy of dominant species in the source ecosystem. Such a transformation could increase negative interactions with co-occurring species or even result in changes to local abiotic conditions.

**Transformative changes in recipient locations (Question 30)**

There are many examples where the movement of species to previously unoccupied areas has caused transformative changes to the community and ecosystem within and beyond the location of introduction, such as during species invasion. Transformative changes could include physical or chemical changes to the habitat and (or) significant alterations to the occurrence or abundance of biota. The mechanisms behind such transformative changes vary based on the species and area that it invades. Although Eastern Sand Darter historically occupied the proposed recipient locations, sufficient time has passed that community dynamics have shifted to a new equilibrium that could be disrupted by its reintroduction. Nevertheless, Eastern Sand Darter lacks the characteristics of common invasive fishes (Olden et al. 2011, Karasov-Olson et al. 2021); specifically, it has a restricted distribution, is in low abundance, has a low tolerance to stressors, and a low rate of dispersal.

**Step 4 – Compile and weigh the scientific evidence**

Scientific knowledge about how to improve the survival or recovery of species listed under SARA is often incomplete, but management decisions must be made despite numerous uncertainties. Management decisions under high uncertainty can be informed by incorporating a structured consensus method with a group of experts (in this case, CSAS participants). During the CSAS meeting, a modified mini-Delphi method was used to gather opinions of participants, and their uncertainty, around questions relevant to the potential reintroduction of Eastern Sand Darter in the Ausable River or Big Otter Creek (Table 1; Table 2) following discussion of the research document (Figure 9). Other factors listed in Figure 2, such as other management objectives or socioeconomic considerations, were not evaluated.

### Modified Mini-Delphi Method

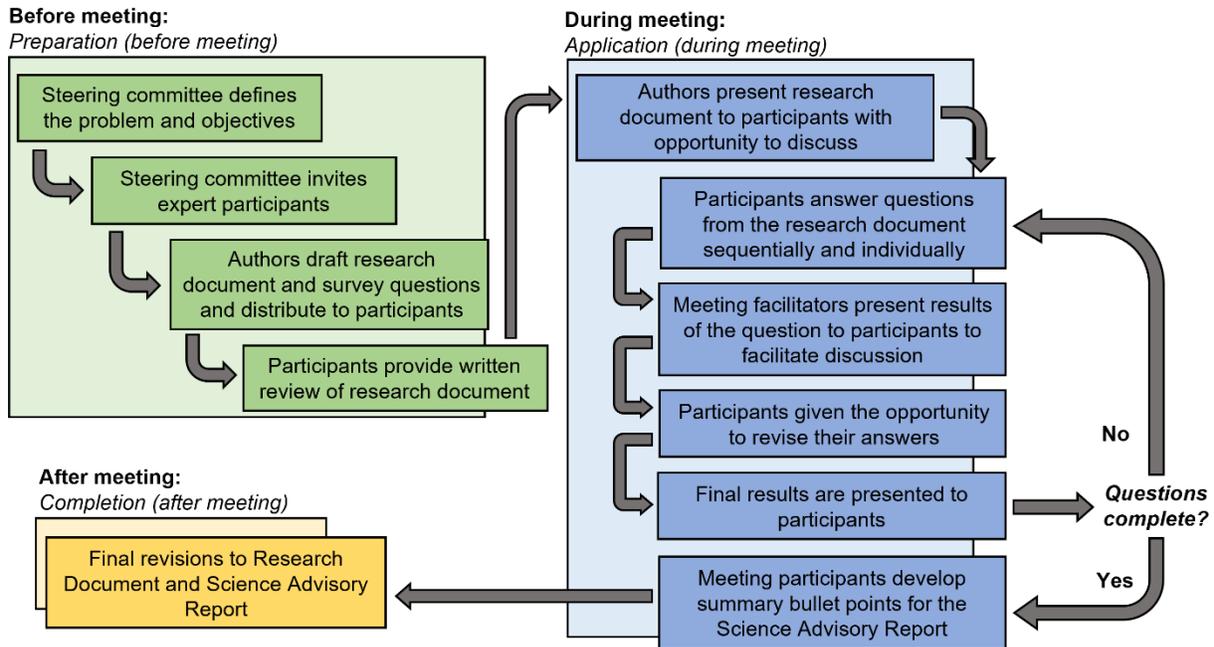


Figure 9. Modified mini-Delphi method to assess the ecological benefits, feasibility, and risks of reintroduction for Eastern Sand Darter. Three stages are outlined for before, during, and after the CSAS meeting.

For each question, participants allocated 100 points between five probability categories to indicate how likely the statement was true while simultaneously providing a level of certainty with that perceived judgement. For example, Question 1a asked participants what the probability is that the Eastern Sand Darter population abundance in the Grand River is greater than 25,000 age-1+ individuals. The participants then allocated 100 points between five categories:

1. Very low probability ( $\leq 5\%$ )
2. Low probability (6–33%)
3. Medium probability (34–66%)
4. High probability (67–94%)
5. Very high probability ( $\geq 95\%$ ).

If a participant felt that the evidence suggested a reasonably high probability that the Grand River population is larger than 25,000 individuals, but had some uncertainty in their answer, they may allocate their points more heavily toward category 4, high likelihood (67–94%). Alternatively, if there was no relevant information about Question 1a and the expert was completely uncertain about the true probability, they may allocate their points uniformly across all categories. This approach allowed the relative certainty of each participant to be incorporated based on their interpretation of the available knowledge base. For each question, participants were asked to answer the question, results were tabulated and presented to the participants,

and then the participants had an opportunity to revise their answers following discussion. The results of the final answers are presented below.

### **Modified Mini-Delphi Results**

Between 15 and 17 experts answered each question from Table 1 and Table 2. Aggregated participant responses to questions and supporting rationales are presented in Colm et al. (2025). There were no questions that had a completely unanimous response (i.e., 100% of points into one category; Figure 10 and 11) suggesting some level of uncertainty with respect to each factor being evaluated. There were two instances where one of the five probability categories received zero votes: Question 1a (Very Low) and Question 12 (Very Low).

The boxplots in Figure 10 and Figure 11 provide summaries of the mini-Delphi results. To generate these plots, a weighted score was calculated for each response to each question per participant. For example, one participant could have allocated 40, 30, 15, 10, and 5 votes to the Very High, High, Medium, Low, and Very Low categories of Question 1a, respectively. Those values were then multiplied by the mid-point probability of the probability category, summed, and divided by 100 to generate a weighted score:

$(Very\ High \times 0.975) + (High \times 0.805) + (Medium \times 0.500) + (Low \times 0.195) + (Very\ Low \times 0.025) = Weighted\ sum/100 = Weighted\ score.$

Using the example:

$$(40 \times 0.975) + (30 \times 0.805) + (15 \times 0.500) + (10 \times 0.195) + (5 \times 0.025) = \frac{72.725}{100} \approx 0.727.$$

The weighted score represents a composite, weighted average of the probability categories, with values closer to 1 representing higher weighted probability and values closer to 0 representing lower weighted probability for each question.

In Figure 10 and Figure 11, the probability category with the most votes (i.e., the mode) was identified with a white square. Asterisks were included in the plots to identify questions where at least one individual provided a score of 20-20-20-20-20, indicating that they were completely uncertain about the true probability. The summed scores scaled to one for each probability category of each question after the final round of questioning are presented in Table A1, and graphically presented in Figures A1–A30 in Colm et al. (2025). The total number of individuals that identified as completely uncertain per question are presented in Table A2 in Colm et al. (2025).

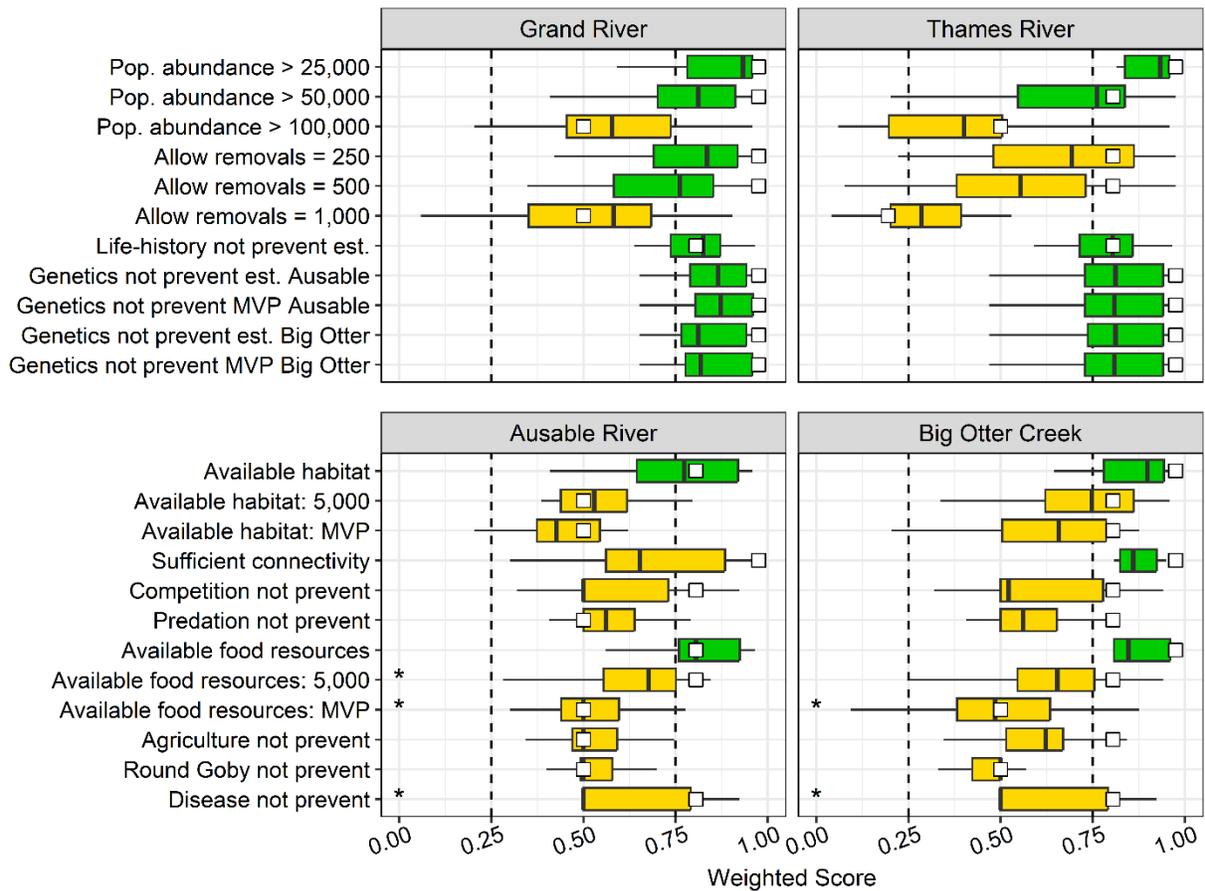


Figure 10. Summary of participant scores for each question in Table 1, which relates to the influence of population, abiotic, biotic, and threat-related factors on reintroduction success. White squares represent the probability category with the most points allocated to it by participants per question. Asterisks indicate that there were one or more participants that identified as being completely uncertain for the question. Yellow boxplots indicate a median score between 0.25 and 0.75 and green boxplots indicate a median score > 0.75. Scores nearing 1.00 suggest that the participants felt that a particular factor had a high probability of being true, therefore supporting reintroduction efforts. MVP = minimal viable population.

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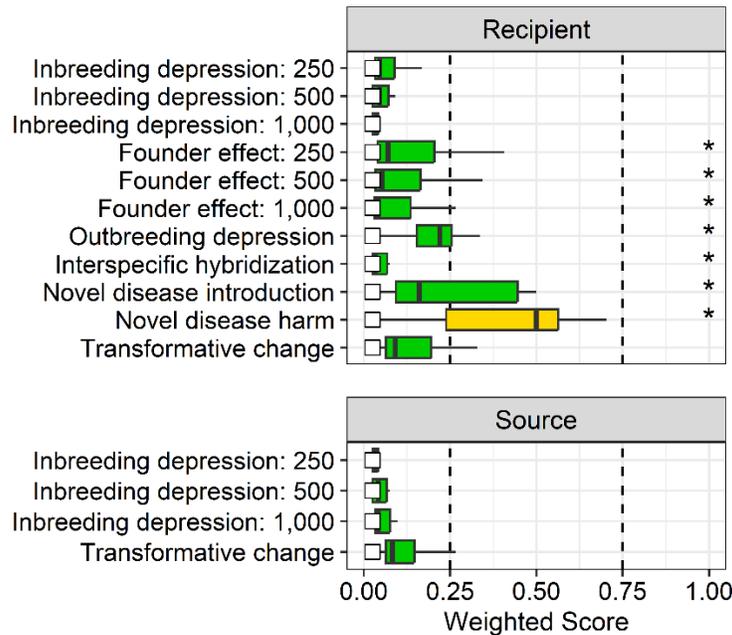


Figure 11. Summary of participant scores for each question in Table 2, which relates to unintended consequences that may occur in the recipient and source location as a result of reintroduction efforts. Squares represent the probability category with the most points allocated to it by participants per question. Asterisks indicate that there were one or more participants that identified as being completely uncertain for the question. Yellow boxplots indicate a median score between 0.25 and 0.75 and green boxplots indicate a median score < 0.25. Scores nearing 0.00 suggest that participants felt that the particular factor had a low probability of occurrence, therefore supporting reintroduction efforts.

**Population, abiotic, biotic, and threat related factors**

The majority of questions about source population factors on reintroduction success had weighted scores > 0.75 (Figure 10). Participant responses suggested a high confidence that genetic diversity, variation, and (or) adaptation would not prevent the establishment of Eastern Sand Darter in the Ausable River or Big Otter Creek, or prevent the population from reaching  $MVP_{99\%}$ , regardless of the source population being considered (Figure 10). There was less certainty among participants about the potential effects of removals on the Grand and Thames river populations, with participants indicating a higher probability of the Grand River population withstanding removals than the Thames River population (Figure 10). The most frequently identified category for whether the Grand River population would be able to withstand removals of 250 and 500 age-1+ females per year for up to 10 years was ‘Very High’, whereas ‘High’ was most frequently identified for the Thames River population. There was less confidence in the ability of the source population to withstand removals of 1,000 age-1+ females per year for up to 10 years in the Grand (mode = Medium) or Thames rivers (mode = Low). Overall, experts felt more confidence that the Grand or Thames rivers could withstand removals of 250 or 500 individuals per year for up to 10 years, and less confidence about removing 1,000 individuals.

Lower scores were given about the influence of abiotic-, biotic-, and threat-related factors on a successful reintroduction (Figure 10). Overall, participant responses indicated a higher probability of suitable abiotic conditions being available for Eastern Sand Darter in Big Otter

Creek than the Ausable River, and this disparity became more noticeable as the reintroduced population size under consideration became larger. When asked about the probability of suitable habitat for Eastern Sand Darter in Big Otter Creek, 85.0% of participant votes were High or Very High whereas 71.2% of votes were Very High or High for the Ausable River. When asked whether there is sufficient suitable habitat to support 5,000 individuals or a population size equal to  $MVP_{99\%}$  in Big Otter Creek, 67.5% and 65.9% of participant votes were for the High or Very High categories, respectively. In comparison, 33.7% and 18.7% of votes were allocated to the High or Very High categories when asked whether there was sufficient suitable habitat in the Ausable River to support 5,000 individuals or a population size equal to  $MVP_{99\%}$ , respectively. Similarly, the respondents identified a higher probability of sufficient connectivity to support an Eastern Sand Darter population in Big Otter Creek than the Ausable River (Figure 10), with 87.7% of scores being High or Very High for Big Otter Creek and 56.8% of scores being High or Very High for the Ausable River.

Participant responses suggested that there was a high probability of appropriate food resources for Eastern Sand Darter in the Ausable River (mode = High) and Big Otter Creek (mode = Very High), but with greater uncertainty as to whether the amount of food is sufficient to support 5,000 individuals (mode = High - both rivers) or a population equal to  $MVP_{99\%}$  (mode = Medium - both rivers; Figure 10). Three participants identified being completely uncertain as to whether the Ausable River or Big Otter Creek contain enough food resources to support an Eastern Sand Darter population at  $MVP_{99\%}$ . Competition and predation were considered to be less limiting in Big Otter Creek than the Ausable River (Figure 10), albeit marginally.

Agriculture and Round Goby were of less concern for supporting the reintroduction of Eastern Sand Darter in Big Otter Creek than the Ausable River (Figure 10). 28.8% of participant votes suggested that the probability of agriculture preventing establishment in the Ausable River was High or Very High (mode = Medium). Similarly, 20.3% of the of participant votes suggested that the probability of Round Goby preventing establishment in the Ausable River was High or Very High (mode = Medium). In comparison, 19.7% of participant votes suggested the probability of agriculture preventing Eastern Sand Darter establishment was High or Very High in Big Otter Creek (mode = Low) and 31.1% of votes suggested the probability of Round Goby preventing establishment was High or Very High (mode = Medium). Note that the modes presented in Figure 10 represent the probability that the threat does not prevent the establishment of Eastern Sand Darter given the particular threat considered. Finally, participants submitted similar answers when asked about the probability of pathogens or parasites restricting the establishment of Eastern Sand Darter in the Ausable River and Big Otter Creek. This included six participants (35.3%) who identified as being completely uncertain for this question (Figure 10).

### Probability of Unintended Consequences

Overall, participants considered the probability of unintended consequences occurring due to reintroduction as Very Low (Figure 11). However, there were more instances where participants identified as being completely uncertain (i.e., 20-20-20-20-20) when considering unintended consequences compared to the factors that influence reintroduction success. The greatest uncertainty was for the potential harm to the recipient freshwater ecosystem that may result from the introduction of a novel pathogen or parasite during reintroduction efforts; although only two people provided scores of 20-20-20-20-20, scores across categories were near 0.200, suggesting significant uncertainty among the participants.

## Sources of Uncertainty

This regional peer-review meeting is the first formal advisory process to use the national guidelines for evaluating the potential benefits and risks of conservation translocation of a freshwater species listed under SARA (Lamothe et al. 2023). The framework helped to identify several uncertainties about the population status, habitat conditions, community dynamics, and risks as they relate to the ability to achieve a successful reintroduction.

First, several source population-related uncertainties emerged that could limit the potential for success, including the lack of definitive population abundance estimates and information on population trajectory. These uncertainties are due, in part, to the lack of long-term monitoring for the species. Indices of population abundance were developed for Eastern Sand Darter using models built with data collected during a single year (2022: Grand River; 2006: Thames River). Uncertainty was compounded by imprecision about the total amount of suitable habitat for Eastern Sand Darter in the Grand and Thames rivers, and the applicability of the modelling results beyond areas that were sampled. In the Grand River, side-scan sonar techniques were unable to differentiate sand from other depositional sediment and sampling for Eastern Sand Darter was only performed in wadable habitats. There was no quantitative information about the total amount of suitable habitat in the Thames River. As a result, population abundance estimates were highly uncertain, with median age-1+ abundance estimates ranging between 41,965–119,159 in the Grand River and 17,030–108,744 in the Thames River, depending on the amount of suitable habitat and under the assumption that models could be extrapolated across wadable and non-wadable habitats. Furthermore, these values represent a single period in time and assume the population trajectory is stable or growing. If these indices of abundance over-estimate the true source population size and/or if the source population is in decline, harm resulting from removals would be greater than reported.

The simulations used to evaluate the potential effects of removal on source populations assumed panmictic populations and that removals for the purposes of reintroduction would occur across the spatial distribution of the species within the population. However, models for the Grand River suggested that abundance of Eastern Sand Darter is not uniform throughout the river, with the Caledonia dam likely restricting movement between river sections. Accordingly, it is unclear how the non-uniform distribution of Eastern Sand Darter in the Grand River would affect estimates of harm from removal efforts. There was less information about the spatial structure of the Thames River population, further emphasizing the uncertainty in population-related considerations for potential source populations.

Models developed to evaluate the probability of achieving reintroduction success used life-history parameters with significant uncertainty and excluded abiotic and biotic variables that may support or hinder the ability to achieve reintroduction objectives. Maximum population growth rate, longevity, age-at-maturity, variation in fecundity, and the rate of survival were all identified as important factors related to estimates of  $MVP_{99\%}$ , the probability of source population harm resulting from removals, and the probability of successful reintroduction. Improving the accuracy of these parameters would reduce the uncertainty around  $MVP_{99\%}$  thresholds and the probability of successful reintroduction efforts. Moreover, model forecasts could be improved by incorporating the potential negative effects of biotic interactions (i.e., competition, predation) and changes in habitat conditions in source and recipient locations. Nevertheless, the inclusion of catastrophes in the population models, which was identified as an important variable across model applications, likely accounted for these potential negative factors.

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Evaluating habitat conditions in potential source and recipient locations was limited by the lack of long-term habitat monitoring and therefore resulted in coarse analyses of habitat suitability. Specifically, measurements were aggregated for potential recipient river systems across two decades of sampling and evaluated at the river or river-segment scale, with only a few instances of measurements and analyses being performed for specific areas being considered for removals or reintroduction. This resulted in uncertainty around how much suitable habitat is available in these systems, the connectivity between habitats, and any spatial or temporal differences in habitat conditions that occur seasonally, annually, or inter-annually.

Quantitative measurements of relevant threats and species responses to those threats were unavailable. As such, it is unclear how the magnitude of threats in source and recipient locations influences the distribution and abundance of Eastern Sand Darter, or would influence reintroduction outcomes. For example, sedimentation and impacts on water quality resulting from historical agricultural practices are identified as the likely cause of extirpation of Eastern Sand Darter in Big Otter Creek, but the species remains extant within rivers that are among the most impacted systems by agriculture in southwestern Ontario (e.g., Thames River, Sydenham River). There is general consensus that agricultural practices as they influence aquatic ecosystem health have improved across southwestern Ontario over time. Nevertheless, the Ausable River and Big Otter Creek are both located in areas with land-use dominated by agriculture and therefore it is unclear how threats related to agriculture may limit the ability to achieve reintroduction objectives.

In addition to agriculture, Round Goby is also considered to be a significant threat to Eastern Sand Darter, but the mechanisms of impact are poorly understood. Research suggests that Eastern Sand Darter and other southern Ontario fishes have shifted and/or narrowed their diet as a result of the Round Goby invasion; nevertheless, the species continue to co-occur in invaded areas. Ultimately, there is uncertainty as to how Round Goby and other co-occurring species may influence establishment of Eastern Sand Darter in potential recipient locations.

The amount of food required to sustain an Eastern Sand Darter population and the amount of food resources available for the species in recipient locations is unknown. Studies have confirmed the presence of commonly consumed prey items for Eastern Sand Darter (Ephemeroptera, Oligochaeta, Chironomidae, Ostracoda, and Cladocera) in the Ausable River and Big Otter Creek, but have yet to evaluate abundance over time. Given that the Ausable River and Big Otter Creek support a similar fish community to the Grand and Thames rivers, and that the macroinvertebrates that comprise the diet of Eastern Sand Darter are present, it is anticipated that sufficient food resources are available for supporting Eastern Sand Darter, but requires more research for confirmation.

Disease occurrence and the potential ecological consequences of disease transfer were identified as the greatest uncertainty regarding reintroduction efforts. There is no published research on the parasite and disease communities in the source and recipient locations, or on parasites or diseases of concern for Eastern Sand Darter. Given the natural movement of fish species among aquatic ecosystems in southwestern Ontario and the potential to spread parasites and disease, it was hypothesized that the parasite and disease communities may be similar between the candidate waterbodies. However, research to evaluate the compositional similarity of parasites and disease between source and recipient locations is the only way to assess the ecological risk from disease transfer.

There was less uncertainty among participants on the potential for genetic effects resulting from the removal and translocation of Eastern Sand Darter from source to recipient locations.

Outbreeding depression was identified as the primary genetics concern, specifically if individuals from the Grand and Thames rivers were used for reintroduction; however, expert participants felt this had a very low probability of influencing reintroduction success. Assuming a single population is sourced for reintroduction efforts, outbreeding depression is unlikely.

The effects of climate change were not explicitly explored in the research document but are relevant to the potential variability in habitat conditions that Eastern Sand Darter may experience in the source and recipient locations. Physiological research related to temperature and oxygen tolerance has been performed to quantify the adaptability of Eastern Sand Darter to different environmental conditions, and has generally demonstrated plasticity in Eastern Sand Darter responses. Nevertheless, the impacts of climate change could limit the probability of reintroduction success depending on the climate scenario (e.g., degree of warming) that is realized, but this was not incorporated into the modelled scenarios.

### **CONCLUSIONS AND ADVICE**

This science advice provides the first application of the national decision support framework for evaluating the suitability of conservation translocation as a recovery action. A Research Document identified the objectives for reintroducing Eastern Sand Darter, assessed the influence of population, abiotic, biotic, and threat-related factors on achieving the objectives, and assessed the probability of unintended consequences for the focal species and ecosystems. A consensus approach was then used to weigh the scientific evidence for how population, abiotic, biotic, and threat-related factors, and unintended consequences, may influence reintroduction outcomes. Overall, Figure 10 and 11 suggest confidence among experts about meeting population, abiotic, biotic, and threat-related factors to achieve reintroduction success, and that the majority of unintended consequences can be avoided. However, the effects of removals on source populations, the amount of suitable habitat, and the potential effects of disease transfer were raised as being among the most uncertain factors influencing a reintroduction outcome. Research to reduce uncertainty around these factors would reduce overall uncertainty associated with the implementation of this recovery action. Should a decision be made to pursue reintroduction for this species, numerous additional science-based considerations were raised during this science advisory meeting, including the importance of developing a suitable experimental design and implementation plan.

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