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**Ontario and Prairie Region**

**Evaluating the Ecological Benefits, Risks, and Feasibility of Reintroduction for  
Eastern Sand Darter (*Ammocrypta pellucida*) in Ontario**

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## Foreword

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

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## TABLE OF CONTENTS

ABSTRACT .....	xi
INTRODUCTION .....	1
1. IDENTIFY OBJECTIVES FOR REINTRODUCTION .....	3
1.1 STATUS OF POTENTIAL SOURCE AND RECIPIENT POPULATIONS .....	3
2. ASSESS THE PROBABILITY OF ACHIEVING THE OBJECTIVES.....	5
2.1. ESTIMATE THE PROBABILITY THAT SUCCESSFUL REINTRODUCTION IMPROVES SURVIVAL OR RECOVERY OF EASTERN SAND DARTER .....	6
Grand River population abundance .....	9
Thames River population abundance .....	19
Potential abundance in the Ausable River and Big Otter Creek .....	22
Modelling the trade-off between removals from the Grand and (or) Thames rivers and the probability of establishment in recipient locations .....	25
2.2. INFORMATION IN SUPPORT OF ESTIMATING THE PROBABILITY OF ESTABLISHING A SELF-SUSTAINING POPULATION OF EASTERN SAND DARTER IN THE RECIPIENT LOCATIONS .....	35
Source population considerations .....	40
Habitat considerations.....	45
Biotic considerations .....	67
Threat considerations.....	70
3. INFORMATION IN SUPPORT OF ESTIMATING THE PROBABILITY OF UNINTENDED CONSEQUENCES RESULTING FROM EASTERN SAND DARTER REINTRODUCTION EFFORTS .....	75
QUESTION 23: INBREEDING DEPRESSION IN THE SOURCE POPULATION .....	76
QUESTION 24: INBREEDING DEPRESSION IN THE RECIPIENT POPULATION .....	76
QUESTION 25: FOUNDER EFFECT IN THE RECIPIENT LOCATION .....	76
QUESTION 26: OUTBREEDING DEPRESSION AS A RESULT OF MIXING GRAND AND THAMES RIVER POPULATIONS .....	77
QUESTION 27: INTERSPECIFIC HYBRIDIZATION IN THE RECIPIENT LOCATION .....	77
QUESTION 28: INTRODUCING DISEASE TO THE RECIPIENT LOCATION .....	78
QUESTION 29: TRANSFORMATIVE CHANGES IN SOURCE LOCATIONS .....	78
QUESTION 30: TRANSFORMATIVE CHANGES IN RECIPIENT LOCATIONS .....	78
APPLICATION .....	79
ACKNOWLEDGEMENTS .....	79
REFERENCES CITED.....	79
APPENDIX I – SUMMARY OF DATA RECORDS.....	90
APPENDIX II – SUMMARY OF FISH SAMPLING EFFORT IN CATFISH CREEK.....	102
APPENDIX III – OVERVIEW OF MODIFIED DELPHI APPROACH.....	103
INTRODUCTION TO THE MODIFIED MINI-DELPHI APPROACH .....	103

---

MODIFIED MINI-DELPHI METHODS .....	104
REFERENCES CITED .....	107

---

## LIST OF FIGURES

Figure 1. Decision support framework outlining science considerations for the use of reintroduction to improve the survival or recovery of freshwater species listed under the Species at Risk Act. Modified from Lamothe et al. (2023). .....	2
Figure 2. Sites with fish and (or) habitat data within Big Otter Creek (purple), Catfish Creek (blue), and the Ausable (yellow), Grand (green), and Thames (red) rivers used in this research document. Built-up area in grey and First Nation areas in tan. ....	4
Figure 3. Square-root transformed extinction probability estimated as the product of simulated population-specific extirpation probabilities. The red curve represents the present-day scenario for Eastern Sand Darter; two populations (pop.) with ‘Good’ status, four with ‘Poor’ status, and two with ‘Unknown’ status (Table 1; Table 2). The blue curve indicates a reintroduction scenario where the reintroduced population is in ‘Poor’ status. The green curve indicates a reintroduction scenario where the reintroduced population is in ‘Good’ status. The purple curve indicates a scenario where an extant ‘Poor’ population improves to ‘Good’ status without reintroduction. The orange curve indicates a scenario where an extant ‘Good’ population declines ‘Poor’ and no reintroduction occurs. The yellow curve indicates a scenario where an extant ‘Good’ population declines to ‘Poor’ and a reintroduction occurs where the reintroduced population has a ‘Poor’ status. The brown curve indicates a scenario where an extant ‘Poor’ population improves to ‘Good’ and a reintroduction occurs where the reintroduced population has ‘Good’ status. ....	8
Figure 4. Areas containing critical habitat for Eastern Sand Darter in the Grand River divided into five sections (Paris [red], Brantford [blue], Middleport [green], Caledonia [orange], and Cayuga [purple]). Farthest upstream detection record (2022) of Round Goby ( <i>Neogobius melanostomus</i> ) indicated with the yellow square. Built-up area in grey and First Nation areas in tan. ....	10
Figure 5. N-mixture model predictions of Eastern Sand Darter detection probability in the Grand River as a function of river velocity (m/s) and river width (m). The solid lines represent the median predictions and the grey areas represent 95% credible intervals. ....	12
Figure 6. N-mixture model predictions of Eastern Sand Darter density (fish/m <sup>2</sup> ) in the Brantford (blue) and Caledonia (red) sections of the Grand River across percent sand and gravel and turbidity measurements (nephelometric turbidity units; NTU). The solid lines represent median predictions and the shaded areas represent 95% credible intervals. Predicted densities in the percent sand and gravel panel incorporate mean turbidity (7.27 NTU) and predicted densities in the turbidity panel incorporate mean percent sand and gravel (82.7%). ....	13
Figure 7. Marginal posterior distribution of site-level Eastern Sand Darter density (fish/m <sup>2</sup> ) in the Brantford (purple) and Caledonia (yellow) sections of the Grand River sampled in 2022, and in the Thames River (grey) sampled in 2006, estimated from independent N-mixture models. ....	14
Figure 8. Indices of age-1+ Eastern Sand Darter population abundance in wadable areas of the Brantford, Middleport, Caledonia, and Cayuga sections of the Grand River based on the proportion of total area with suitable habitat (i.e., areas with at least 50% sand and fine gravel substrate; colours) and model-generated densities (Figure 7). Note the different scales of the x-axis. ....	16
Figure 9. Indices of age-1+ Eastern Sand Darter population abundance in non-wadable areas of the Brantford, Middleport, Caledonia, and Cayuga sections of the Grand River based on the proportion of total area with suitable habitat (i.e., areas with at least 50% sand and fine gravel substrate; colours) and model-generated densities (Figure 7). Note the different scales of the x-axis. ....	17

---

Figure 10. N-mixture model predictions of Eastern Sand Darter detection probability in the Thames River as a function of the proportion of a site consisting of woody debris and boulders (i.e., cover; x-axis). The solid line represents the median prediction and the grey area represents the 95% credible interval.....	20
Figure 11. N-mixture model predictions of Eastern Sand Darter density (fish/m <sup>2</sup> ) in the Thames River across proportion (prop.) of sand and fine gravel and water clarity (cm) measurements. Model built using data from Dextrase (2013). The solid lines represent the median predictions and the grey areas represent 95% credible intervals. Predicted densities in the proportion of sand and gravel panel incorporate mean clarity (26.7 cm) and predicted densities in the clarity panel incorporate mean proportion of sand and fine gravel (0.456). .....	21
Figure 12. Indices of age-1+ abundance of the Thames River Eastern Sand Darter population in wadable sections and overall (Total) based on the proportion of the total area with suitable habitat (i.e., areas with at least 50% sand and fine gravel substrate; colours) and model-generated densities (Figure 7). .....	22
Figure 13. The division of sections in the Ausable River. Colours indicate the upstream (red) and middle river sections (blue). Farthest upstream detection record (2017) of Round Goby ( <i>Neogobius melanostomus</i> ) indicated with yellow square. Built-up area in grey and First Nation areas in tan. ....	23
Figure 14. Potential age-1+ abundance of a reintroduced Eastern Sand Darter population in the Ausable River and Big Otter Creek based on the proportion of total area with suitable habitat (i.e., areas with at least 50% sand and fine gravel substrate; 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. ....	24
Figure 15. Generalized life cycle used to model Eastern Sand Darter population dynamics. <i>F<sub>i</sub></i> represents stage-specific annual fertility, <i>S<sub>i</sub></i> represents stage-specific survival rate, and <i>P<sub>3</sub></i> represents the probability of surviving and remaining in stage 3. ....	26
Figure 16. The probably of extirpation for Eastern Sand Darter as a function of age-1+ female population size. The rug plot indicates extirpation occurrences. The solid line represents the logistic regression mean trend with 95% confidence intervals (grey area) where the probability of a catastrophic event ranges from 5% to 20% per generation and maximum population growth rate ranges from 1.56 to 2.69. The dashed line indicates a 1% probability of extirpation. ....	30
Figure 17. Probability of reducing age-1+ female Eastern Sand Darter abundance below the mean minimum viable population ( <i>MVP</i> <sub>99%</sub> ) size (i.e., probability of removal impacts). ....	31
Figure 18. Probability of age-1+ female abundance in the recipient location being above the lower 95% confidence interval (CI) of estimated minimum viable population size ( <i>MVP</i> <sub>99%</sub> ; 3,098 age-1+ females) and mean <i>MVP</i> <sub>99%</sub> (16,651 age-1+ females) for Eastern Sand Darter in the Ausable River and Big Otter Creek after reintroductions cease (i.e., probability of success) as a function of the total number of age-1+ females individuals reintroduced. ....	33
Figure 19. von Bertalanffy growth curve for Eastern Sand Darter generated using data from the lower Thames River collected in 2006 and 2007. Total length is calculated using the formula: $TL_{mm} = 55.521 - e^{-1.59Age + 0.47}$ (Finch et al. 2013). ....	42
Figure 20. Length-frequency distribution of Eastern Sand Darter collected from the Grand ( <i>n</i> = 3,630 individuals; 2007-2022; pink) and Thames ( <i>n</i> = 997 individuals; 2006-2022; purple) rivers by DFO (2013) and Dextrase (2013). Note the differences in scales of the y-axis between panels. ....	43

---

---

Figure 21. A source population selection framework for conservation translocation that requires knowledge of abiotic and biotic requirements for the species being considered. Originally presented in Lamothe et al. (2023). .....	44
Figure 22. Conceptual relationship of habitat comparisons between source locations of Eastern Sand Darter (blue) and candidate locations for reintroduction (yellow). Scenario 1: Mean and variance match; Scenario 2: Mean match, variance mismatch; Scenario 3: Mean mismatch, variance match; Scenario 4: Mean and variance mismatch. ....	45
Figure 23. Representation of habitat niche for juvenile (< age-1) and adult (age-1+) Eastern Sand Darter. Blue and green indicate adult and juvenile life-stages, respectively.....	47
Figure 24. Minimum area for population viability (MAPV; km <sup>2</sup> ) of Eastern Sand Darter in the Brantford and Caledonia sections of the Grand River, and the Thames River. Dashed lines indicate median values. Dark grey indicates 95% confidence interval. ....	48
Figure 25. Percent sand and silt measurements at sites in the middle and upper sections of the Ausable River, and in the Grand and Thames rivers where Eastern Sand Darter was captured. Points indicate empirical measurements and are jittered for visual purposes. Violins (colours) were scaled proportionally to the number of observations per location. Sample sizes per variable per location are presented in Table 15.....	53
Figure 26. Percent sand and fine gravel, percent silt and clay, and mean substrate size (mm) at sites in the Ausable River and Big Otter Creek, and in the Grand and Thames rivers where Eastern Sand Darter was captured. Points indicate measurements from Dextrase (2013) and are jittered for visual purposes. Violins (colours) were scaled proportionally to the number of observations per location. Two observations were removed from the mean substrate size plot that were > 80 mm, one from the Ausable River and one from the Grand River. Sample sizes per variable per location are presented in Table 17.....	53
Figure 27. Histogram and kernel density plots for habitat measurements in the middle (yellow) and upper (grey and black) sections of the Ausable River, and at sites in the Grand (orange) and Thames (blue) rivers where Eastern Sand Darter was captured. Horizontal segments on the top of each plot indicate range of measured values per waterbody. Sample sizes per variable per location are presented in Table 15. ....	54
Figure 28. Histogram and kernel density plots for habitat measurements in the Ausable River (yellow) and Big Otter Creek (green), and in the Grand (orange) and Thames (blue) rivers where Eastern Sand Darter was captured. Horizontal segments on the top of each plot indicate range of measured values per waterbody. Plots only include data from Dextrase (2013). Sample sizes per variable per location are presented in Table 17.....	55
Figure 29. Percent sand and silt measurements at sites in Big Otter Creek, and in the Grand and Thames rivers where Eastern Sand Darter was captured. Points indicate empirical measurements and are jittered for visual purposes. Violins (colours) were scaled proportionally to the number of observations per waterbody. Sample sizes per variable per waterbody are presented in Table 20. ....	60
Figure 30. Substrate composition as the proportion of total weight of sample from historical (left) and targeted (right) sites in Big Otter Creek, Ontario (2020). Substrate categories: silt (< 0.063 mm; red), very fine sand (0.063–0.125 mm; orange), fine sand (0.125–0.25 mm; yellow), medium sand (0.25–0.50 mm; green), coarse sand (0.5–1.0 mm; blue), very coarse sand (1.0–2.0 mm; purple), very fine gravel (2.0–4.0 mm; grey), fine gravel (4.0–8.0 mm; black), and medium gravel (≥ 8 mm; white). Recreated from Barnucz et al. (2022).....	61

---

---

Figure 31. Relative proportion of total weight of medium gravel, sand and fine gravel, and silt at one site in the Grand River and 12 sites in Big Otter Creek (six historical and six targeted). Red dots indicate actual measurements and were jittered horizontally for visual purposes..... 62

Figure 32. Histogram and kernel density plots for habitat measurements in Big Otter Creek (green) and at sites in the Grand River (orange) and Thames River (blue) where Eastern Sand Darter was captured. Sample sizes per variable per waterbody are presented in Table 20. Plots include all available data. Horizontal segments on the top of each plot indicate range of measured values per river system. .... 63

Figure 33. Daily mean water temperature measurements from the Grand River (orange; Cockshutt Bridge), Thames River (blue; upstream of Springbank Dam), and Big Otter Creek (green; downstream of Eden Road Bridge and off John Pound Road) between June 2019 and August 2021. Horizontal bars represent monthly (June-November) mean  $CT_{max} \pm 95\%$  confidence interval for Eastern Sand Darter from the Grand River (Firth et al. 2021a). .... 65

Figure 34. Mean monthly water temperature measurements from the Grand River (orange; Cockshutt Bridge), Thames River (blue; upstream of Springbank Dam), and Big Otter Creek (green; downstream of Eden Road Bridge and off John Pound Road) between June 2019 and August 2021. .... 65

Figure 35. Conceptual diagram representing the negative effects of the most relevant threats (grey) to Eastern Sand Darter growth, survival, and reproduction in southwestern Ontario. Red arrows indicate anticipated effects of the threat on the particular life-history process. Black arrows indicate the relationship between survival, growth, and reproduction such that impacts to survival can lead to changes in reproduction. .... 70

Figure 36. Alkalinity (mg/L), conductivity ( $\mu\Omega$ ), pH, sodium (mg/L), and turbidity (Formazine Turbidity Unit; FTU) measurements (y-axis) by month (colour) across years (x-axis) from sites in Sylvan, Springbank, and Exeter, ON. .... 72

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## LIST OF TABLES

Table 1. Relative abundance index, population trajectory, and status of Eastern Sand Darter populations in southwestern Ontario, sorted by drainage. Numbers in parentheses indicate certainty rankings, listed as: 1 = quantitative analysis, 2 = catch per unit effort or standardized sampling, or 3 = expert opinion. Table modified from DFO (2012) and originally from Bouvier and Mandrak (2010).	5
Table 2. Theoretical probability of extinction [Pr(ext)] for scenarios with differing numbers of populations with Good, Poor, or Unknown status. Theoretical extirpation probabilities over the next 100 years per population status were assumed to be: Good = 1–5%, Poor = 25–50%, or Unknown = 25–50%.	7
Table 3. Summary of N-mixture model results for the Grand River, including the mean estimate with standard deviation (SD) and the lower and upper 95% credible interval (LCI–UCI).	12
Table 4. Goodness-of-fit statistics (Bayesian p-values) for detection and abundance components of N-mixture models developed for the Grand and Thames rivers.	13
Table 5. Values used to estimate suitable habitat in the Grand River based on river segments measured (Brantford, Caledonia) or not measured (Middleport, Cayuga) in areas of critical habitat in the Grand River in 2022. Note: n = number of samples used to calculate average width.	15
Table 6. Median and mean indices of abundance for age-1+ Eastern Sand Darter in wadable, non-wadable, and combined (total; bold) habitats in the Grand River by river section and the proportion of suitable habitat ( $\geq 50\%$ sand and fine gravel). Minimum totals represent total abundance when the proportion of suitable habitat is at the minimum values considered per section and maximum totals represent total abundance when the proportion of suitable habitat is at the maximum values considered.	18
Table 7. Summary of N-mixture model results for the Thames River, including the mean estimate with standard deviation (SD) and the lower and upper 95% credible interval (LCI–UCI).	20
Table 8. Median and mean indices of abundance for age-1+ Eastern Sand Darter in wadable, non-wadable, and combined (total; bold) habitats in the Thames River and the proportion of total area with suitable habitat (i.e., areas with at least 50% sand and fine gravel substrate).	22
Table 9. Median potential abundance of age-1+ Eastern Sand Darter in the Ausable River and Big Otter Creek based on the density estimates from the Grand and Thames rivers and the proportion of total area with suitable habitat (i.e., areas with at least 50% sand and fine gravel substrate).	24
Table 10. Parameter values incorporated in the matrix population model for Eastern Sand Darter. Fixed parameters were held constant across model runs and uncertain parameters were sampled randomly from the specified distribution.	25
Table 11. Results of the Eastern Sand Darter minimum viable population size (MVP) logistic regression. Variables were transformed to z-scores prior to modeling. K = carrying capacity; Prcat = probability of a catastrophic event; $\lambda_{max}$ = maximum population growth rate; Tmat = age-at-maturity; SE = standard error; p = significance level.	29
Table 12. Logistic regressions of the probability of removal impacts on source populations. Variables were scaled and centered prior to modeling. $\log_{10}N_a$ = log-10 transformed initial female density; Prcat = probability of a catastrophic event; CVf = clutch size coefficient of	

---

variation ;  $\lambda_{\max}$  = maximum population growth rate; S = age-1+ survival rate; SE = standard error; p = significance level. .... 30

Table 13. Logistic regressions of reintroduction success. Variables were scaled and centered prior to modeling. MVP99% LCI = Minimum viable population size (99%) lower credible interval (3,098 age-1+ females); log10A = log10-transformed total number of additions; log10K = log10-transformed carrying capacity; M= translocation mortality;  $\lambda_{\max}$  = maximum population growth rate; Tmat = age-at-maturity; S = age-1+ survival rate; Prcat = probability of a catastrophic event. Tmax = longevity; CVf = clutch size coefficient of variation; SE = standard error; p = significance level..... 32

Table 14. 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. MVP99% = Minimum viable population size (99%). ..... 36

Table 15. Results of Fligner-Killeen’s (FK) tests for homogeneity of variance of habitat measurements from the middle and upper sections of the Ausable River, and sites where Eastern Sand Darter was captured in the Grand and Thames rivers. Included are tests for when all data are included. Note: n = number of samples and p = significance level. .... 51

Table 16. Results of Kruskal-Wallis chi-square (KW  $\chi^2$ ) rank sum test of habitat measurements from the middle and upper sections of the Ausable River and sites where Eastern Sand Darter was captured in the Grand and Thames rivers. .... 51

Table 17. Results of Fligner-Killeen’s (FK) tests for homogeneity of variance of habitat measurements from the Ausable River, Big Otter Creek, and sites where Eastern Sand Darter was captured in the Grand and Thames rivers. Included are tests for when only data from Dextrase (2013) are included. Sample sizes (n) are provided for each variable per waterbody. Note: p = significance level. .... 52

Table 18. Results of Kruskal-Wallis chi-square (KW  $\chi^2$ ) rank sum test of habitat measurements from the Ausable River, Big Otter Creek, and sites where Eastern Sand Darter was captured in the Grand and Thames rivers. Mean  $\pm$  SD (standard deviation) presented. Included are tests for when only data from Dextrase (2013) are included. Sample sizes are presented in Table 17. Note: p = significance level. .... 52

Table 19. Percent overlap in kernel density estimates for habitat variables in the middle and upper sections of the Ausable River with measurements taken in the Grand and Thames rivers where Eastern Sand Darter was captured. .... 55

Table 20. Results of Fligner-Killeen’s (FK) tests for homogeneity of variance of habitat measurements from Big Otter Creek and sites where Eastern Sand Darter was captured in the Grand and Thames rivers. Included are tests for when all data are included. Note: n = number of samples and p = significance level. .... 59

Table 21. Results of Kruskal-Wallis chi-square (KW  $\chi^2$ ) rank sum test of habitat measurements from Big Otter Creek and sites where Eastern Sand Darter was captured in the Grand and Thames rivers. Mean  $\pm$  SD (standard deviation) presented. Included are tests for when all data are included. Sample sizes are presented in Table 20. Note: p = significance level. .... 59

Table 22. Percent overlap in kernel density estimates for habitat variables in Big Otter Creek with measurements taken in the Grand and Thames rivers where Eastern Sand Darter was previously detected. .... 63

Table 23. Ecological risk considerations for the focal taxa and other ecosystem components in source and recipient habitats of proposed reintroductions. .... 75

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## ABSTRACT

Federal recovery strategies frequently describe the need for species reintroductions to improve the survival and recovery of species listed under the *Species at Risk Act* (SARA); however, there have been relatively few attempts to reintroduce SARA-listed freshwater species. The limited reintroduction effort has been partially attributed to incomplete ecological knowledge of candidate species and uncertainty about how to assess the ecological benefits, risks, and feasibility of reintroduction. Reintroduction is proposed as a recovery measure for Eastern Sand Darter (*Ammocrypta pellucida*) because the species has experienced several extirpations across its Canadian range. Knowledge of Eastern Sand Darter ecology has improved over the past 15 years, and recent national guidance describes the scenarios in which reintroduction could be effective as a recovery tool, which together provide an opportunity to evaluate the potential use of reintroduction to improve recovery. The objective of this document was to use a decision-support framework to evaluate the potential benefits, risks, and feasibility of an experimental Eastern Sand Darter reintroduction in Ontario. Information on the ecological factors that could influence the success of reintroduction is presented, including indices of abundance for potential source populations, analyses to estimate change in the extinction risk of Eastern Sand Darter given the re-establishment of a population, an evaluation of habitat suitability and threats, and risks of harm for Eastern Sand Darter and the broader ecosystem at source and recipient locations. Overall, the information provided in this document can be used by the Species at Risk Program to determine if the benefits of 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 species.

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## INTRODUCTION

Eastern Sand Darter (*Ammocrypta pellucida*) is a small (< 8 cm), benthic fish species with a disjunct distribution in Ontario and Québec. The species was originally listed under the *Species at Risk Act* (SARA) in July 2003 as a single Designatable Unit (DU), but given the geographic separation between populations in Ontario and Québec, these populations were recognized as separate DUs in 2013 (DFO 2012, 2014). The subsequent discovery of Eastern Sand Darter in West Lake, Ontario (Reid and Dextrase 2014, 2017) and new information on genetic structure (Walter et al. 2022) led the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) to recommend that the Ontario DU be split into a Southwestern Ontario DU (Threatened) and West Lake DU (Threatened), with the Québec DU maintaining its Special Concern status (COSEWIC 2022). The Southwestern Ontario DU includes all populations formerly identified under the Ontario DU besides West Lake and is the focus of this research document.

In support of the long-term recovery objective of restoring self-sustaining populations to formerly occupied habitats, the recovery strategy for the (former) Ontario DU identified the need to investigate the feasibility of reintroduction (DFO 2012). Species reintroduction is defined as the intentional movement and release of an organism to a location within its native range from which it has disappeared (IUCN/SSC 2013). Similar to other freshwater fish species in Canada (Lamothe and Drake 2019, Lamothe et al. 2019a), reintroduction has not been undertaken for Eastern Sand Darter, at least partially due to incomplete ecological knowledge of the species and uncertainty about how to assess the ecological benefits, risks, and feasibility of the recovery action.

A national Canadian Science Advisory Secretariat (CSAS) process recently developed a decision-support framework that can be used to provide guidance around the scenarios in which reintroduction could be used as a recovery tool for freshwater fish and mussel species listed under SARA (Figure 1; Lamothe et al. 2023). Application of this framework provides decision-makers the ecological information about how reintroduction could benefit a particular SARA-listed species and the ecological risks of performing such an action. The five-step decision-support framework is used here to evaluate the benefits, risks, and feasibility of an Eastern Sand Darter reintroduction in Ontario. Below, an overview of the five steps is provided followed by a summary of how each step is addressed in this document.

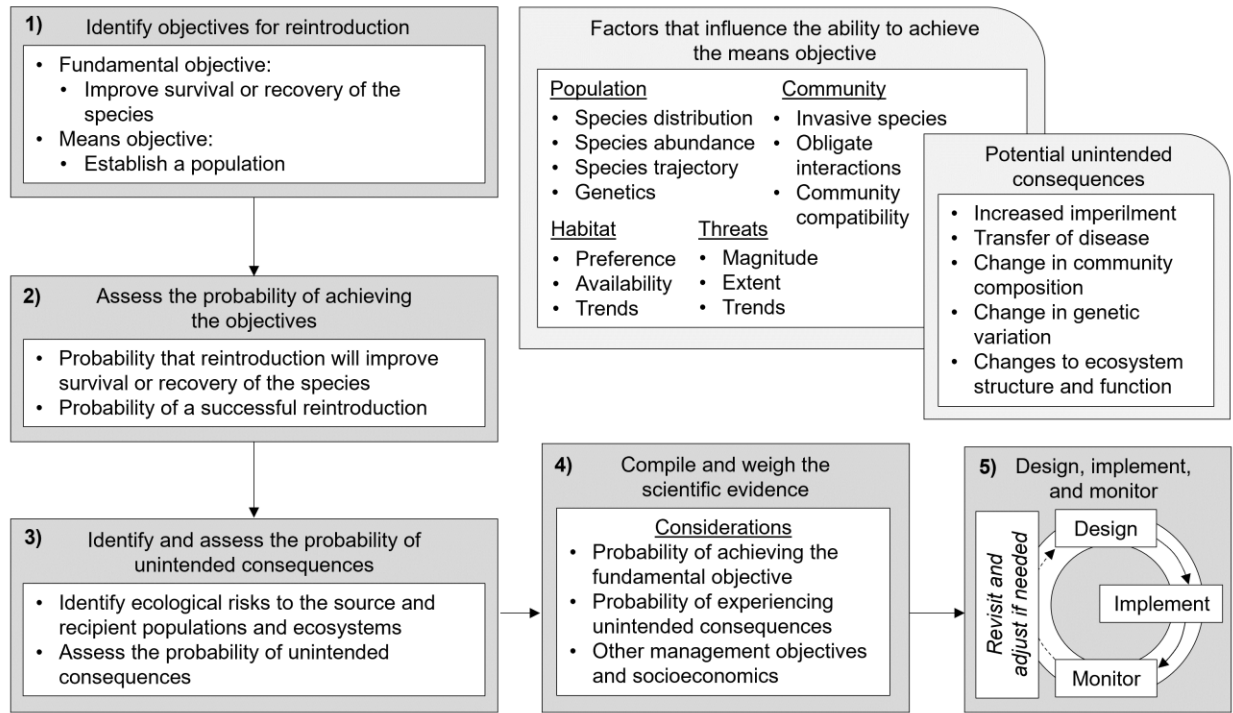


Figure 1. Decision support framework outlining science considerations for the use of reintroduction to improve the survival or recovery of freshwater species listed under the Species at Risk Act. Modified from Lamothe et al. (2023).

The first step in the decision-support framework is to identify the objectives for performing a reintroduction. For SARA-listed freshwater fishes, the fundamental objective of any recovery action is to improve the survival or recovery of the species. Reintroduction implies improving survival and recovery through the establishment of a population in a formerly occupied location, which requires defining the focal populations and the temporal and spatial scale of management. The next step is to assess the probability that reintroduction will be successful and lead to improved survival or recovery of the species (Step 2; Figure 1); neither of these outcomes are certain. In the case of reintroduction, this requires identifying the likelihood that the reintroduction will be successful and the potential harm of removing individuals from wild source populations. Along with the need to evaluate the probability of achieving the objectives is the need to identify and assess risks of unintended consequences (Step 3; Figure 1). Species reintroduction could have unintended consequences on the focal species and/or other ecosystem components and therefore careful evaluation of risk is needed. In Steps 4 and 5, the information generated in the first three steps is weighed against other management objectives and socioeconomic factors. If the decision is made to perform a reintroduction, protocols must be established for implementation, including monitoring to detect changes to the focal species, non-focal species, and other ecosystem components. Steps 1–3 are critically assessed in this document, with Steps 4 and 5 to be considered following a review of compiled evidence.

This document is structured to follow the decision-support framework (Figure 1). In the first section, objectives for reintroducing Eastern Sand Darter are described with emphasis on identifying candidate source and recipient populations. In Step 2, simulations are first presented to evaluate the benefit of an additional population to the long-term persistence of the species. *N*-mixture models were then built to generate an index of population abundance for potential source populations and matrix population models were used to understand the effects of

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removals on source population persistence and the probability of establishing a reintroduced population. After the modeling sections, questions are presented to identify how additional population, habitat (abiotic and biotic), and threat factors may influence the ability to achieve a successful reintroduction, and answered using the best available empirical information. This included the use of habitat-matching analyses to evaluate the similarity of habitat characteristics between sites where Eastern Sand Darter was previously detected and potential recipient locations, comparisons of fish communities between source and recipient locations, and the potential effects of agricultural threats and Round Goby on reintroduction success. Following the models and considerations in Step 2, questions are presented in Step 3 that consider the potential risks of unintended consequences of reintroduction. Ultimately, the information gained through this process can support evidence-based decisions about implementing Eastern Sand Darter reintroductions in southwestern Ontario.

## 1. IDENTIFY OBJECTIVES FOR REINTRODUCTION

The first step in assessing reintroduction for Eastern Sand Darter is to develop a problem statement and identify the fundamental and means objectives of performing the management action (Figure 1):

- **Problem statement:** Extirpations of Eastern Sand Darter have occurred in at least three Ontario rivers: Ausable River, Big Otter Creek, and Catfish Creek. The most recent records of Eastern Sand Darter in these systems are from 1928 (Ausable River), 1941 (Catfish Creek), and 1955 (Big Otter Creek). The causes of extirpation are generally unknown as data are unavailable for the historical period. Extirpation is hypothesized to have been caused by habitat degradation related to excess siltation resulting from historical agricultural practices, including the channelization of stream habitats (primarily in the Ausable River; Holm and Mandrak 1996, DFO 2012, Dextrase 2013). Species reintroduction has been identified as a potential strategy for improving the survival and recovery of Eastern Sand Darter in Ontario (DFO 2012). Two extant populations in Ontario (Grand and Thames rivers) are potentially suitable for sourcing individuals for reintroduction, owing to their high relative abundance (DFO 2011). There is less certainty around the most suitable recipient location for reintroduction; therefore, all three rivers with extirpations are initially evaluated.
- **Fundamental objective:** Improve the survival or recovery of Eastern Sand Darter in southwestern Ontario.
- **Means objective:** Reintroduction to a formerly occupied location is the approach being considered to improve the survival and recovery of Eastern Sand Darter in southwestern Ontario.

Assessing the means objective requires first identifying potential source population(s) and recipient locations for the reintroduction, and evaluating how reintroduction efforts, focused on the population level, could improve survival or recovery of the species. A brief review of the status of potential source populations and recipient locations for an Eastern Sand Darter reintroduction is provided. This is followed by analyses that evaluate the potential improvements in survival for Eastern Sand Darter from a successful reintroduction and its ecological feasibility.

### 1.1 STATUS OF POTENTIAL SOURCE AND RECIPIENT POPULATIONS

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. As well, populations outside of southwestern Ontario may experience different conditions than those in the potential recipient locations.

The two candidate source populations (Grand and Thames rivers) are the only populations considered to be in relatively good condition compared to other extant populations in Canada (Table 1; DFO 2012). There is extensive habitat and fish community data from the Grand and Thames rivers for the 2002–2022 period (Figure 2). These data are primarily stored in databases managed by Fisheries and Oceans Canada at the Great Lakes Laboratory for Fisheries and Aquatic Sciences, Burlington, Ontario (Table A1; Figure 2; [Fish Biodiversity Database](#)), and include data from several projects that were performed in direct support of Eastern Sand Darter management and recovery efforts (Drake et al. 2008, Finch 2009, Finch et al. 2011, 2013, 2018, Dextrase 2013, Dextrase et al. 2014, Burbank et al. 2019, Firth et al. 2021a,b, Barnucz et al. 2022, Firth et al. 2023).

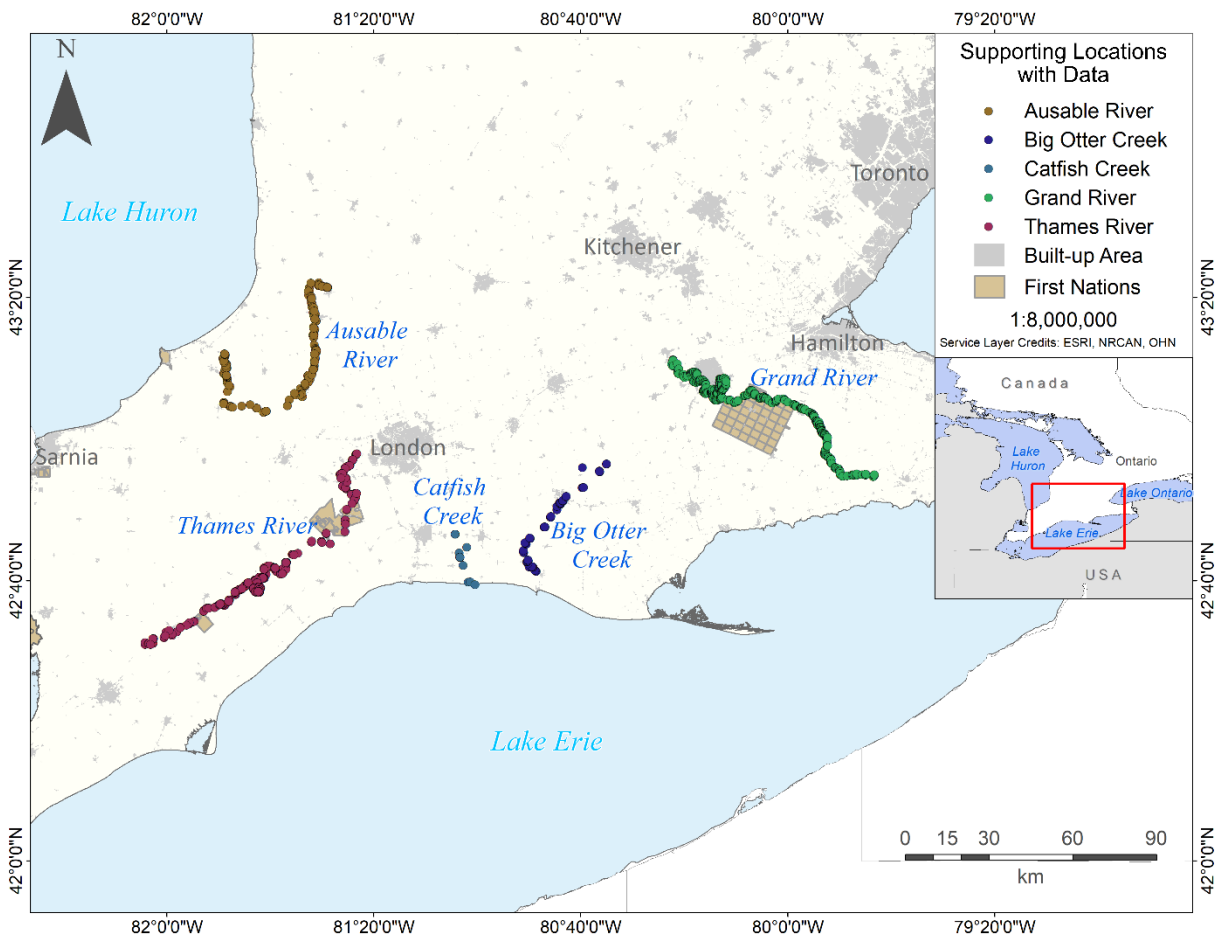


Figure 2. Sites with fish and (or) habitat data within Big Otter Creek (purple), Catfish Creek (blue), and the Ausable (yellow), Grand (green), and Thames (red) rivers used in this research document. Built-up area in grey and First Nation areas in tan.

Table 1. Relative abundance index, population trajectory, and status of Eastern Sand Darter populations in southwestern Ontario, sorted by drainage. Numbers in parentheses indicate certainty rankings, listed as: 1 = quantitative analysis, 2 = catch per unit effort or standardized sampling, or 3 = expert opinion. Table modified from DFO (2012) and originally from Bouvier and Mandrak (2010).

Drainage	Population	Relative abundance index	Population trajectory	Population status
Lake Huron	Ausable River	Extirpated (2)	Not applicable (2)	Extirpated
Lake St. Clair	Lake St. Clair	Low (2)	Declining (3)	Poor
Lake St. Clair	Thames River	High (1)	Stable (1)	Good
Lake St. Clair	Sydenham River	Low (2)	Unknown (3)	Poor
Lake Erie	Western Basin	Unknown (3)	Unknown (3)	Unknown
Lake Erie	Rondeau Bay	Unknown (3)	Unknown (3)	Unknown
Lake Erie	Long Point Bay	Low (2)	Declining (2)	Poor
Lake Erie	Catfish Creek	Extirpated (3)	Not applicable (3)	Extirpated
Lake Erie	Big Otter Creek	Extirpated (3)	Not applicable (3)	Extirpated
Lake Erie	Big Creek	Low (3)	Unknown (3)	Poor
Lake Erie	Grand River	High (2)	Stable (2)	Good

Only three fish surveys were available for Catfish Creek (one of the three extirpated populations; Table A1 **Error! Reference source not found.**) with very little associated habitat or water-quality measurements (see Appendix 2 for a summary of the data). Due to these data limitations and qualitative evidence of unsuitable water clarity and substrate, Catfish Creek is not being considered as a candidate location for Eastern Sand Darter reintroduction. In contrast, significant fish community data have been collected in the Ausable River and Big Otter Creek (Table A1). There were 197 data records available for the Ausable River, between the north end of ‘The Cut’ to Exeter, Ontario (2002–2022) and 75 data records for Big Otter Creek between Vienna and Otterville, Ontario (2018–2021; Figure 1). Given the knowledge of the systems and available data, the Ausable River and Big Otter Creek are identified as the two candidate locations to be evaluated for Eastern Sand Darter reintroduction.

## 2. ASSESS THE PROBABILITY OF ACHIEVING THE OBJECTIVES

The next step in the decision-support framework is to assess the probability of achieving the identified objectives (Figure 1). For reintroduction, this involves assessing how removals could change the viability of source populations (DFO 2012) and the degree to which the establishment of a new population could improve the status and viability of the species in Canada. Below, analyses are presented to:

1. Assess the probability that achieving a successful reintroduction would improve survival and recovery of the species; and,
2. Assess the probability of achieving a successful reintroduction.

Although it is identified in Step 3 (Figure 1), an analysis of the potential harm of removals is included in Step 2 as it is an outcome of the modelling performed for evaluating the probability of achieving a successful reintroduction.

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## 2.1. ESTIMATE THE PROBABILITY THAT SUCCESSFUL REINTRODUCTION IMPROVES SURVIVAL OR RECOVERY OF EASTERN SAND DARTER

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 can demonstrate how the number of populations, and the persistence of those populations, affects long-term species viability. There are currently eight<sup>1</sup> populations of Eastern Sand Darter in southwestern Ontario; two populations in 'Good' condition (Grand River, Thames River), 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; Table 1). The probability of persistence for each population is unknown. For the purposes of illustration, assume that the extirpation probability of populations with a 'Good' status is 1–5% over the next 100 years, 25–50% for populations with a 'Poor' status, and 25–50% for populations with an 'Unknown' status. If the probability of extirpation is not correlated among populations, extinction probability (i.e., extinction of the southwestern Ontario DU) is the product of population extirpation probabilities.

A statistical resampling procedure can be used to generate extinction probability distributions where population-specific extirpation probabilities are randomly selected from uniform distributions with bounds based on population status (i.e., Good, Poor, or Unknown). Using the parameters described above and assuming independence among populations, the median probability of extinction in the present-day scenario with eight populations is  $1.86 \times 10^{-6}$  (Table 2; Figure 3). A ninth population can be added to represent a reintroduction, where the reintroduced population could be in 'Good' or 'Poor' condition, depending on the ecological outcome. Relative to the present-day scenario, the addition of a ninth population (i.e., reintroduction) in 'Good' condition would reduce median extinction probability by 97.20% ( $5.22 \times 10^{-8}$ ) while a ninth population in 'Poor' condition would reduce median extinction probability by 63.09% ( $6.87 \times 10^{-7}$ ; Table 2). Therefore, reductions in overall extinction risk are expected to occur with a successful reintroduction, with the expected magnitude based on the outcome of the reintroduced population.

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<sup>1</sup> A single Eastern Sand Darter was detected in the Detroit River in 2013, which may constitute a ninth population (COSEWIC 2022), but for the purposes of this example is not included.

Table 2. Theoretical probability of extinction [ $Pr(ext)$ ] for scenarios with differing numbers of populations with Good, Poor, or Unknown status. Theoretical extirpation probabilities over the next 100 years per population status were assumed to be: Good = 1–5%, Poor = 25–50%, or Unknown = 25–50%.

Scenario	Good	Poor	Unknown	Total populations	$Pr(ext)$	Percent change in $Pr(ext)$ relative to present-day
Present-day	2	4	2	8	$1.86 \times 10^{-6}$	-
Reintroduce Poor population	2	5	2	9	$6.87 \times 10^{-7}$	-63.09%
Reintroduce Good population	3	4	2	9	$5.22 \times 10^{-8}$	-97.20%
Poor population improves to Good status without reintroduction	3	3	2	8	$1.43 \times 10^{-7}$	-92.29%
Good population declines to Poor status without reintroduction	1	5	2	8	$2.52 \times 10^{-5}$	1,255.25%
Good population declines to Poor status and a Poor population is reintroduced	1	6	2	9	$9.24 \times 10^{-6}$	396.60%
Poor population improves to Good status and a Good population is reintroduced	4	3	2	9	$3.91 \times 10^{-9}$	-99.79%

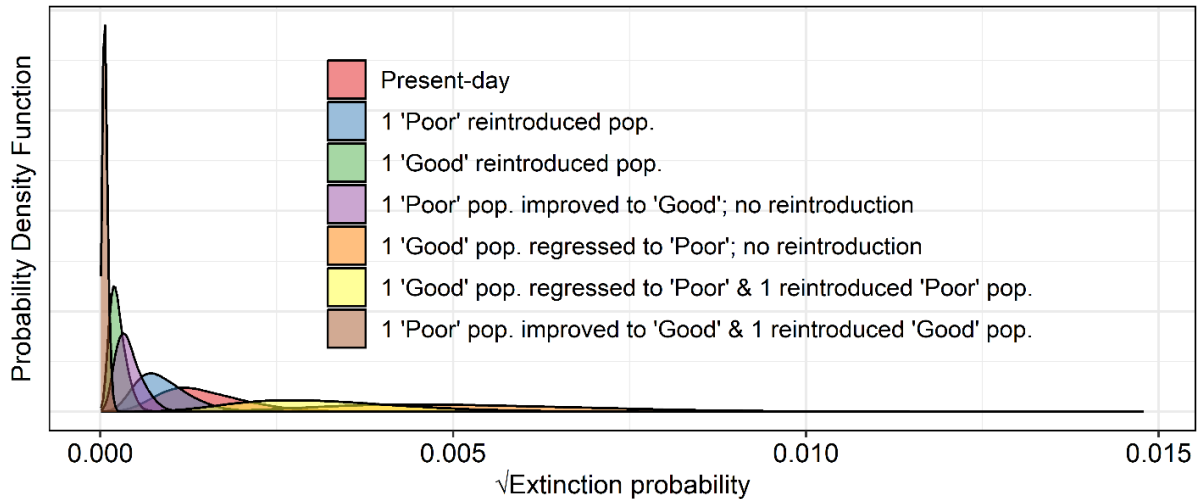


Figure 3. Square-root transformed extinction probability estimated as the product of simulated population-specific extirpation probabilities. The red curve represents the present-day scenario for Eastern Sand Darter; two populations (pop.) with 'Good' status, four with 'Poor' status, and two with 'Unknown' status (Table 1; Table 2). The blue curve indicates a reintroduction scenario where the reintroduced population is in 'Poor' status. The green curve indicates a reintroduction scenario where the reintroduced population is in 'Good' status. The purple curve indicates a scenario where an extant 'Poor' population improves to 'Good' status without reintroduction. The orange curve indicates a scenario where an extant 'Good' population declines 'Poor' and no reintroduction occurs. The yellow curve indicates a scenario where an extant 'Good' population declines to 'Poor' and a reintroduction occurs where the reintroduced population has a 'Poor' status. The brown curve indicates a scenario where an extant 'Poor' population improves to 'Good' and a reintroduction occurs where the reintroduced population has 'Good' status.

Scenarios could occur where an extant population with 'Poor' status improves to 'Good' status by means of recovery and habitat restoration actions other than reintroduction, which would result in a 92.29% decrease in median extinction probability ( $1.43 \times 10^{-7}$ ) relative to the present-day scenario (Table 2). Alternatively, owing to threats or other factors and unrelated to reintroduction, scenarios could arise where a 'Good' extant population declines to 'Poor' over time (i.e., 1 'Good', 5 'Poor', and 2 'Unknown'), which would result in a 1,255.25% increased median extinction probability ( $2.52 \times 10^{-5}$ ) relative to the present-day, no-reintroduction situation (Table 2; Figure 3). A subsequent reintroduction of a 'Poor' population (i.e., 1 'Good', 6 'Poor', and 2 'Unknown') would result in a median extinction probability of  $9.24 \times 10^{-6}$  (Table 2). In this situation, the reintroduced population decreases overall extinction risk relative to a scenario where reintroduction does not occur, but the effect is insufficient to provide a net decrease in extinction probability relative to the declining extant population. Finally, a scenario could occur where an extant population with 'Poor' status improves to 'Good' and a reintroduction occurs where the reintroduced population is in 'Good' condition (i.e., 4 'Good', 3 'Poor', and 2 'Unknown'), which could result in a 99.79% reduction in extinction probability relative to the present-day scenario (Table 2).

It is unlikely that population extirpation probabilities are uncorrelated. Large-scale habitat changes (e.g., reduced water availability; Round Goby invasion) can act simultaneously on Eastern Sand Darter across its southwestern Ontario range. As a result, extinction probability among extant populations is likely higher than in the provided example. If population extirpation rates are perfectly correlated, then the species would go extinct following the extirpation of the population with the lowest probability of extirpation, all else being equal. Determining the probability of extirpation for each extant and reintroduced population would help to refine the

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overall improvement in species viability associated with reintroduction. The example presented here indicates that the reintroduction of a self-sustaining population could improve species viability.

In the next sections, models are presented to estimate the abundance of Eastern Sand Darter in the Grand and Thames rivers based on capture data and estimated habitat availability. In addition, the potential future abundance of reintroduced populations in the Ausable River or Big Otter Creek are estimated based on habitat availability estimates and extrapolation of model-estimated Eastern Sand Darter densities from the Grand and Thames rivers. Population viability analyses were then used to understand how removing individuals from the Grand or Thames river populations would influence the viability of either source population across different removal scenarios. Further, population models were used to estimate the probability of establishing a self-sustaining population of Eastern Sand Darter in recipient habitats based on the frequency and magnitude of additions and the amount of suitable habitat available for the species.

### **Grand River population abundance**

Sampling in the Grand River was conducted in 2022 to estimate the population density of Eastern Sand Darter (Gáspárdy et al. 2025). Fish and habitat surveys occurred at sites (10 m x 10 m) with  $\geq 50\%$  coverage of sand and fine gravel substrate to increase the chance of detecting Eastern Sand Darter. Prior to sampling, 125 random points were selected between Wilkes Dam (Brantford) and Cayuga (Figure 4), though the 25-km stretch of habitat upstream of Caledonia Dam (Middleport section in Figure 4) was avoided given the lower likelihood of detecting Eastern Sand Darter in this area (Dextrase 2013). At each randomly selected point, field crews located the closest area of at least 10 m x 10 m habitat that contained at least 50% sand and fine gravel substrate. If there was no site with  $\geq 50\%$  sand and fine gravel substrate within a 200 m radius of the randomly selected point, field crews moved on to the next randomly selected site.

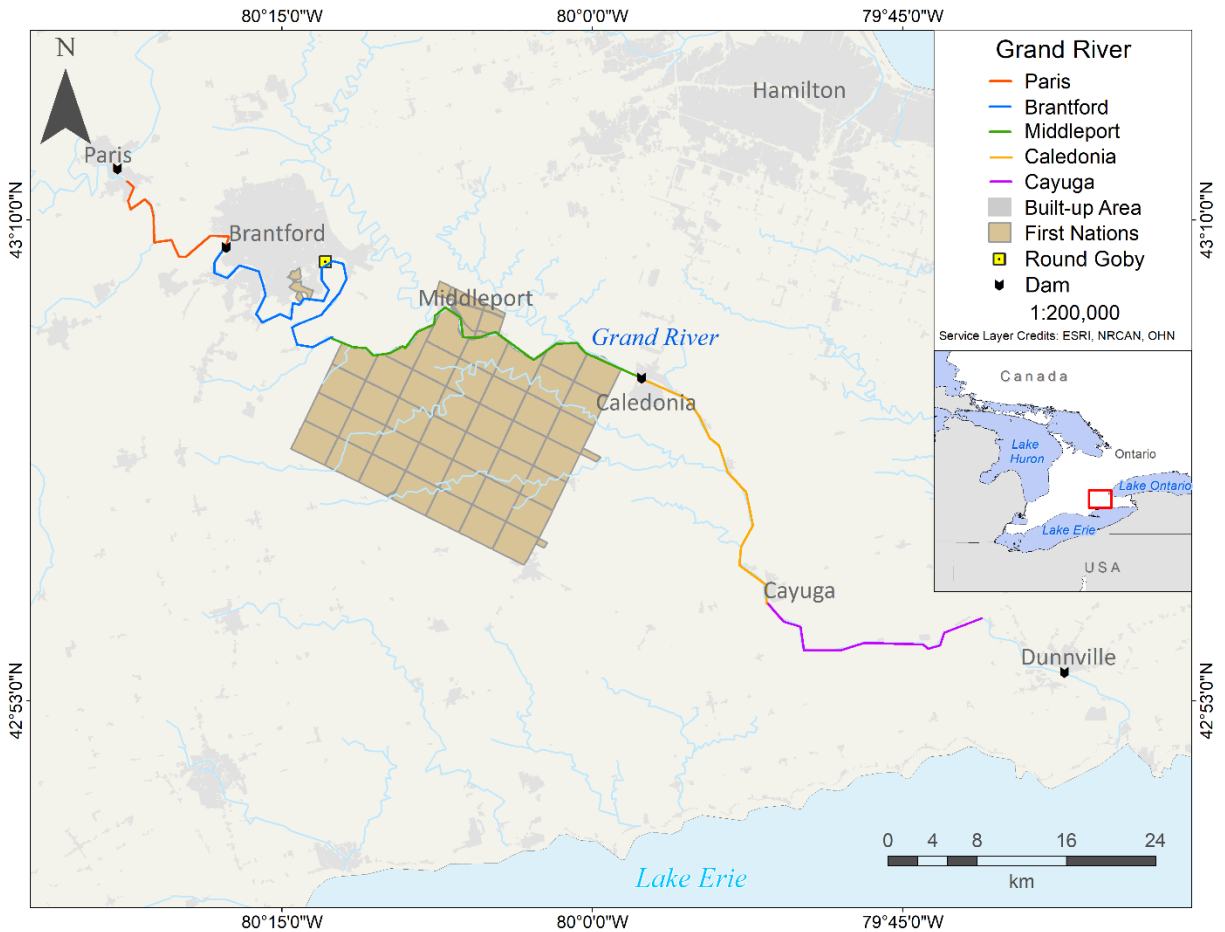


Figure 4. Areas containing critical habitat for Eastern Sand Darter in the Grand River divided into five sections (Paris [red], Brantford [blue], Middleport [green], Caledonia [orange], and Cayuga [purple]). Farthest upstream detection record (2022) of Round Goby (*Neogobius melanostomus*) indicated with the yellow square. Built-up area in grey and First Nation areas in tan.

Fishes were captured using a 9.14 m long by 1.8 m tall bag seine with 3 mm mesh. Three seine hauls were performed at each site in a downstream direction. After each seine haul, captured Eastern Sand Darter were placed in separate bins for processing and enumerated per haul. Fish were returned to the site following completion of all three seine hauls. An assessment of habitat was performed after fish sampling that included documenting the percent cover of substrate using a modified Wentworth substrate classification (Bain 1999), and recording water temperature ( $^{\circ}\text{C}$ ), conductivity ( $\mu\text{S}/\text{cm}$ ), dissolved oxygen ( $\text{mg}/\text{L}$ ), and turbidity (NTU; Gáspárdy et al. 2025).

One hundred sites were sampled, with 54 in the Brantford section and 46 in the Caledonia section (Figure 4). In total, 610 Eastern Sand Darter were captured, with 0–174 individuals detected per site. All Eastern Sand Darter captured in the 2022 sampling were deemed to be adults (age 1+) based on the observed length distribution. Observed site density across all sites was  $0.0635 \text{ fish}/\text{m}^2$ , with a mean of  $0.0994 \text{ fish}/\text{m}^2$  in the Brantford section and  $0.0174 \text{ fish}/\text{m}^2$  in the Caledonia section. Eastern Sand Darter was not detected at 62 sites (64.5%). Modeling presented in the next section includes 96 of the 100 sampled sites, with four sites excluded due to missing covariate data.

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## Grand River N-mixture model methods

Data collected in 2022 from the Grand River were fit with  $N$ -mixture models to estimate ‘true’ Eastern Sand Darter site abundance ( $N$ ) in each system while accounting for detection probability ( $p$ ; Royle 2004, Kéry and Royle 2015).  $N$ -mixture models consist of abundance and detection sub-models that can include covariates at the site and survey level. The model structure used to estimate Eastern Sand Darter abundance was:

$$C_{ij}|N_i \sim \text{Multinomial}(N_i, \pi(p_{ij})) \quad \text{Equation (1)}$$

$$N_i \sim \text{NB}(\mu_i, k) \quad \text{Equation (2)}$$

$$\text{logit}(p_{ij}) = \alpha_0 + a_1 RV_i + a_2 RW_i + u_{ij} \quad \text{Equation (3)}$$

$$\text{log}(\lambda_i) = \beta_0 + \beta_1 \text{Section}_i + \beta_2 SG_i + \beta_3 \text{Turb}_i, \quad \text{Equation (4)}$$

where  $C_{ij}$  is Eastern Sand Darter catch at site  $i$  during survey  $j$ . Detection probability at a site for a given survey was conditional on the encounter history from the previous survey due to the depletion survey design (Gáspárdy et al. 2025). As such, the detection probability model was fit using a multinomial distribution with cell probabilities ( $\pi$ ) calculated as the product of detection probabilities during sample  $j$  and the probability of not being detected in the previous samples [e.g.,  $\pi_3 = (1 - p_1)(1 - p_2)p_3$ ]. A sample-specific random effect ( $u_{ij}$ ) was included to allow for excess variability in detection among samples (i.e., overdispersion), where  $u_{ij} \sim \mathcal{N}(0, \sigma_{\text{sample}})$ . The abundance model was fit using a negative binomial ( $NB$ ) distribution because of similar overdispersion evident in preliminary analyses;  $\mu$  is the expected site abundance and  $k$  is the size parameter for the negative binomial distribution.

Habitat characteristics previously identified as important for modelling Eastern Sand Darter abundance were included as covariates for the detection ( $a$ ) and abundance ( $\beta$ ) processes (Dextrase 2013). River velocity ( $RV$ ; m/s) and river width ( $RW$ ; m) were used as covariates for the detection process, while percent sand and gravel substrate ( $SG$ ), turbidity ( $Turb$ ; NTU), and river section (Brantford = 1; Caledonia = 0; Figure 4) were included in the abundance model.

Model fit was assessed using a Bayesian  $p$ -value approach where a fit statistic was estimated for the observed data as well as simulated data obtained from the posterior predictive distributions (Gelman et al. 1996). The Bayesian  $p$ -value describes the proportion of the observed fit statistic that exceeds the fit statistic for the simulated data with a value close to 0.5 indicating good fit [e.g.,  $Pr(\text{fit}_{\text{obs}} > \text{fit}_{\text{sim}})$ ]. Three fit statistics were used:

1.  $\chi^2 = \sum (y - E(y))^2 / E(y)$  Equation (5)

2.  $\text{Freeman-Tukey} = \sum (\sqrt{y} - \sqrt{E(y)})^2$  Equation (6)

3.  $\text{Sum of squares} = \sum (y - E(y) / \sigma_y)^2$  Equation (7)

Model fit was assessed using each fit statistic for both the detection and abundance sub-models. In addition, a Bayesian  $p$ -value was used to confirm that the model produced an appropriate number of zeros. The  $N$ -mixture model was fit using NIMBLE in R version 4.2.2 with the ‘nimble’ package (de Valpine et al. 2017, 2022a, b, R Core Team 2022). Vague priors were used for all model parameters.

## Grand River N-mixture model results

Detection probability of Eastern Sand Darter individuals was generally high in the Grand River (mean = 0.520; 95% Confidence Interval [CI] = 0.354–0.668) but decreased with increasing river width and river velocity (Figure 5). High variability was observed in detection probability between

seine hauls; excess variability was accounted for with  $\sigma_{sample} = 0.91$  (Table 2), suggesting substantial overdispersion.

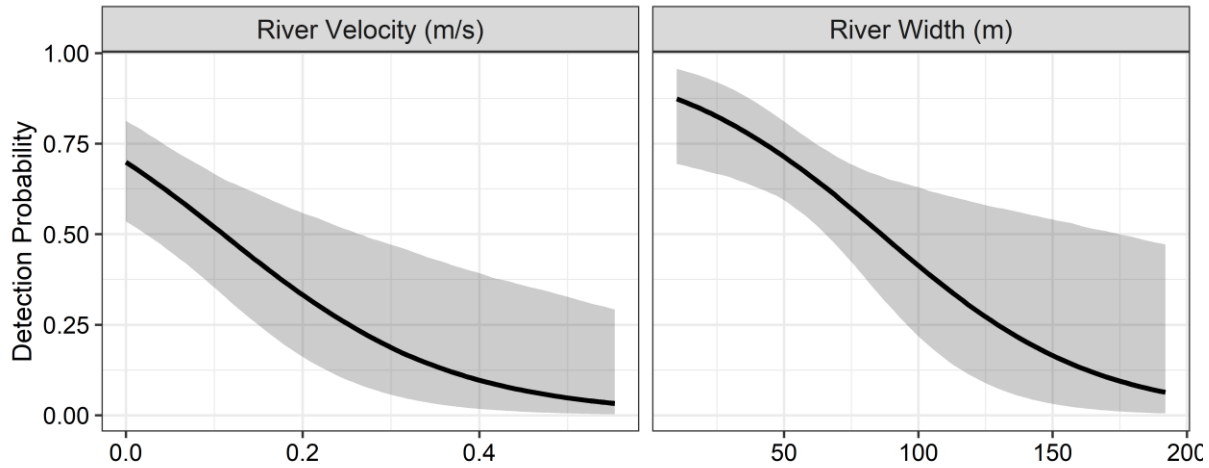


Figure 5. *N*-mixture model predictions of Eastern Sand Darter detection probability in the Grand River as a function of river velocity (m/s) and river width (m). The solid lines represent the median predictions and the grey areas represent 95% credible intervals.

Model-estimated mean site density was low, with greater density estimates in the Brantford section than the Caledonia section (Table 3); mean density was 0.0191 fish/m<sup>2</sup> (95% CI: 0.0079–0.0535) in the Caledonia section and 0.0754 fish/m<sup>2</sup> (95% CI: 0.0359–0.1811) in the Brantford section. Mean density estimates correspond to sites with mean percent sand and gravel (82.7%; range: 50–100%) and turbidity (7.27 NTU; range: 1.10–47.03 NTU). Site density tended to be higher at sites with a high percent of sand or gravel and low turbidity (Figure 6). The small size parameter for the negative binomial distribution (Table 3) indicates that density was variable across sites.

Table 3. Summary of *N*-mixture model results for the Grand River, including the mean estimate with standard deviation (SD) and the lower and upper 95% credible interval (LCI–UCI).

Sub-model	Parameters	Symbol	Mean	SD	LCI	UCI
Detection	Intercept	$\alpha_0$	0.07	0.32	-0.60	0.70
Detection	River velocity (m/s)	$\alpha_1$	-0.81	0.26	-1.32	-0.29
Detection	River width (m)	$\alpha_2$	-1.03	0.41	-1.85	-0.25
Detection	Dispersion parameter	$\sigma_{sample}$	0.91	0.19	0.58	1.34
Abundance	Intercept	$\beta_0$	0.65	0.49	-0.24	1.68
Abundance	River section (Brantford)	$\beta_1$	1.37	0.62	0.14	2.59
Abundance	% Sand and gravel	$\beta_2$	1.14	0.39	0.39	1.93
Abundance	Turbidity (NTU)	$\beta_3$	-1.09	0.59	-2.29	0.04
Abundance	Dispersion parameter	$\mu$	0.16	0.03	0.10	0.23

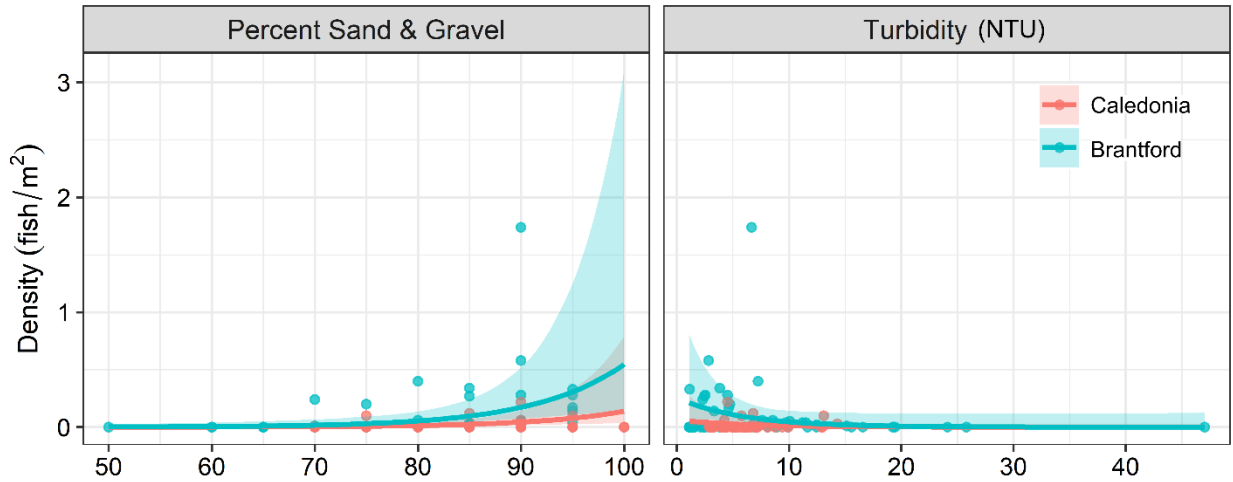


Figure 6. *N*-mixture model predictions of Eastern Sand Darter density (fish/m<sup>2</sup>) in the Brantford (blue) and Caledonia (red) sections of the Grand River across percent sand and gravel and turbidity measurements (nephelometric turbidity units; NTU). The solid lines represent median predictions and the shaded areas represent 95% credible intervals. Predicted densities in the percent sand and gravel panel incorporate mean turbidity (7.27 NTU) and predicted densities in the turbidity panel incorporate mean percent sand and gravel (82.7%).

There was no indication of a lack-of-fit in either the detection or abundance sub-models across all fit statistics once overdispersion was accounted for (Table 4). As well, an appropriate number of 0s was predicted from the model fit relative to what was observed (Table 4). Estimates of site-level density were made for river sections independently, where averages of turbidity (7.27 NTU) and percent sand and gravel (82.6%) were used to make predictions (Figure 7).

Table 4. Goodness-of-fit statistics (Bayesian *p*-values) for detection and abundance components of *N*-mixture models developed for the Grand and Thames rivers.

River	Model	$\chi^2$	Freeman-Tukey	Sum of Squares	Proportion of Zeros
Grand River	Detection	0.454	0.547	0.530	NA
Grand River	Abundance	0.466	0.396	0.519	0.733
Thames River	Detection	0.503	0.501	0.448	NA
Thames River	Abundance	0.445	0.532	0.474	0.499

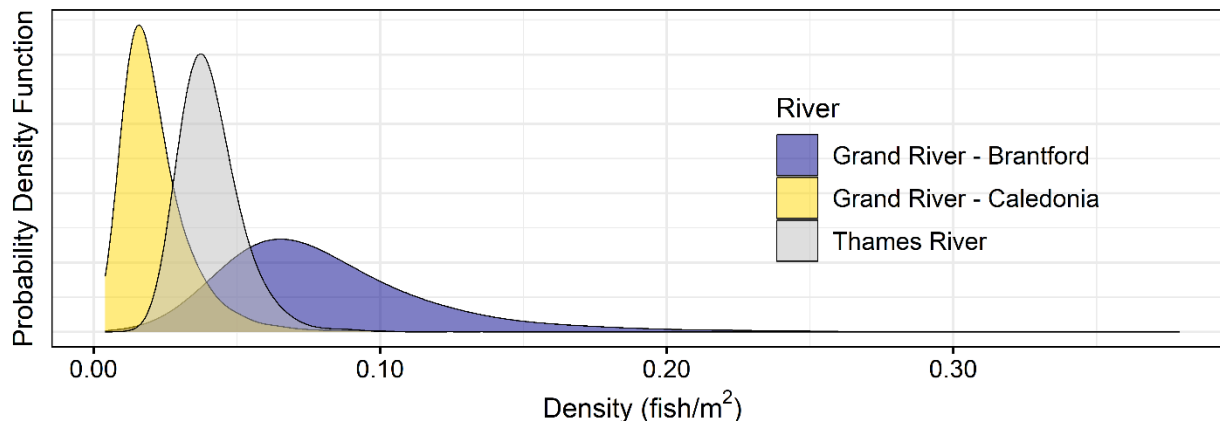


Figure 7. Marginal posterior distribution of site-level Eastern Sand Darter density (fish/m<sup>2</sup>) in the Brantford (purple) and Caledonia (yellow) sections of the Grand River sampled in 2022, and in the Thames River (grey) sampled in 2006, estimated from independent *N*-mixture models.

### Grand River population abundance

An index of age-1+ population abundance was calculated for Eastern Sand Darter based on mean density estimates from the *N*-mixture model and the estimated amount of suitable habitat. First, the total available aquatic habitat was estimated by dividing the area in the Grand River containing Eastern Sand Darter critical habitat into five sections to accommodate the 2022 sampling design (Figure 4). Eastern Sand Darter sampling and site-level habitat measurements were conducted in two of the five sections in 2022 (Brantford, Caledonia), whereas the remaining three sections were not sampled in 2022 (Paris, Middleport, and Cayuga; Figure 4). Eastern Sand Darter has never been detected upstream of Wilkes dam in Brantford, so the Paris segment of critical habitat was not included in the assessment of habitat availability.

For the remaining four sections, total available habitat was estimated as the length of the critical habitat polygon multiplied by the mean river width obtained from field measurements in that section (Table 5). This is an overestimate of suitable habitat for Eastern Sand Darter; as such, total available habitat estimates were reduced based on an estimate of the proportion of the total habitat that contained at least 50% sand and fine gravel substrate cover, the habitat characteristic frequently associated with species occupancy (Dextrase et al. 2014). The amount of sand and fine gravel substrate in the Grand River was estimated using side-scan sonar surveys completed in 2020 (Caledonia section) and 2022 (Brantford section; Milne Technologies 2023). The Ontario Ministry of Natural Resources performed the surveys in the Caledonia section using a Humminbird® Helix™ 9 sonar unit while Fisheries and Oceans Canada performed the surveys in Brantford section using a Humminbird® Helix™ 7 sonar unit. Survey data were evaluated by Milne Technologies (2023) with QTC SwathView waveform analysis using a Klein 3000/3900 model, the SonarWiz seabed classification tool, and manually to delineate substrate patches. Based on the surveyed locations, fine sediment, sand, and rippled sand, covered approximately 80.3% of the Brantford section and 31.6% of the Caledonia section.

Table 5. Values used to estimate suitable habitat in the Grand River based on river segments measured (Brantford, Caledonia) or not measured (Middleport, Cayuga) in areas of critical habitat in the Grand River in 2022. Note:  $n$  = number of samples used to calculate average width.

River segment	Segment length (m)	Average width (m)	$n$	Total available habitat (m <sup>2</sup> )
Brantford	29,058	66.40	166	1,929,451
Middleport	25,007	120.77	49	3,020,095
Caledonia	19,520	106.48	95	2,078,490
Cayuga	16,945	154.10	10	2,611,225
<b>Total</b>	-	-	<b>332</b>	<b>9,639,261</b>

An index of age-1+ abundance for Eastern Sand Darter was calculated for:

1. wadable habitat only (i.e., < 1.2 m depth); and,
2. the entire area of critical habitat based on estimates of suitable habitat.

As the  $N$ -mixture models were developed using data from wadable habitats, an additional assumption was made that those densities could be extrapolated to non-wadable areas. Expert judgement was used to determine the proportion of wadable habitat in each river section; approximately 30% of the Brantford, 10% of the Middleport, 50% of the Caledonia, and 10% of the Cayuga sections were considered to be wadable (Jason Barnucz, DFO, pers. comm.). Furthermore, due to the inability to differentiate fine sediment (i.e., silt) from sand in the sonar data, the amount of suitable habitat (i.e., sites with at least 50% sand and fine gravel) was assumed to be between 20% and 65% of the total estimated habitat in the Brantford section and between 5% and 25% of the total estimated habitat in the Middleport, Caledonia, and Cayuga sections.

A statistical resampling approach was used to generate the index of abundance for Eastern Sand Darter. Random draws were made from the marginal posterior distribution of mean density for the Brantford and Caledonia sections (Figure 7), multiplied by random draws from a distribution of the assumed proportion of occupied suitable habitat, and finally multiplied by the amount of total available habitat. A uniform distribution between 0.20 and 0.65 was used for estimating occupied suitable habitat in the Brantford section, while a uniform distribution between 0.05 and 0.25 was used for the Middleport, Caledonia, and Cayuga sections. Brantford density estimates were used for estimating age-1+ abundance in the Brantford section while Caledonia density estimates were used for estimating age-1+ abundance in the Middleport, Caledonia, and Cayuga sections as there is a general understanding that there are lower densities of Eastern Sand Darter downstream of the Brantford section. This resampling approach was repeated 20,000 times to generate a distribution of prospective age-1+ Eastern Sand Darter population sizes for each river section in wadable and non-wadable habitats. The result of the resampling procedure is a distribution of abundances for Eastern Sand Darter in the area that contains critical habitat (downstream of the Wilkes Dam) in the Grand River (Figure 8, Figure 9).

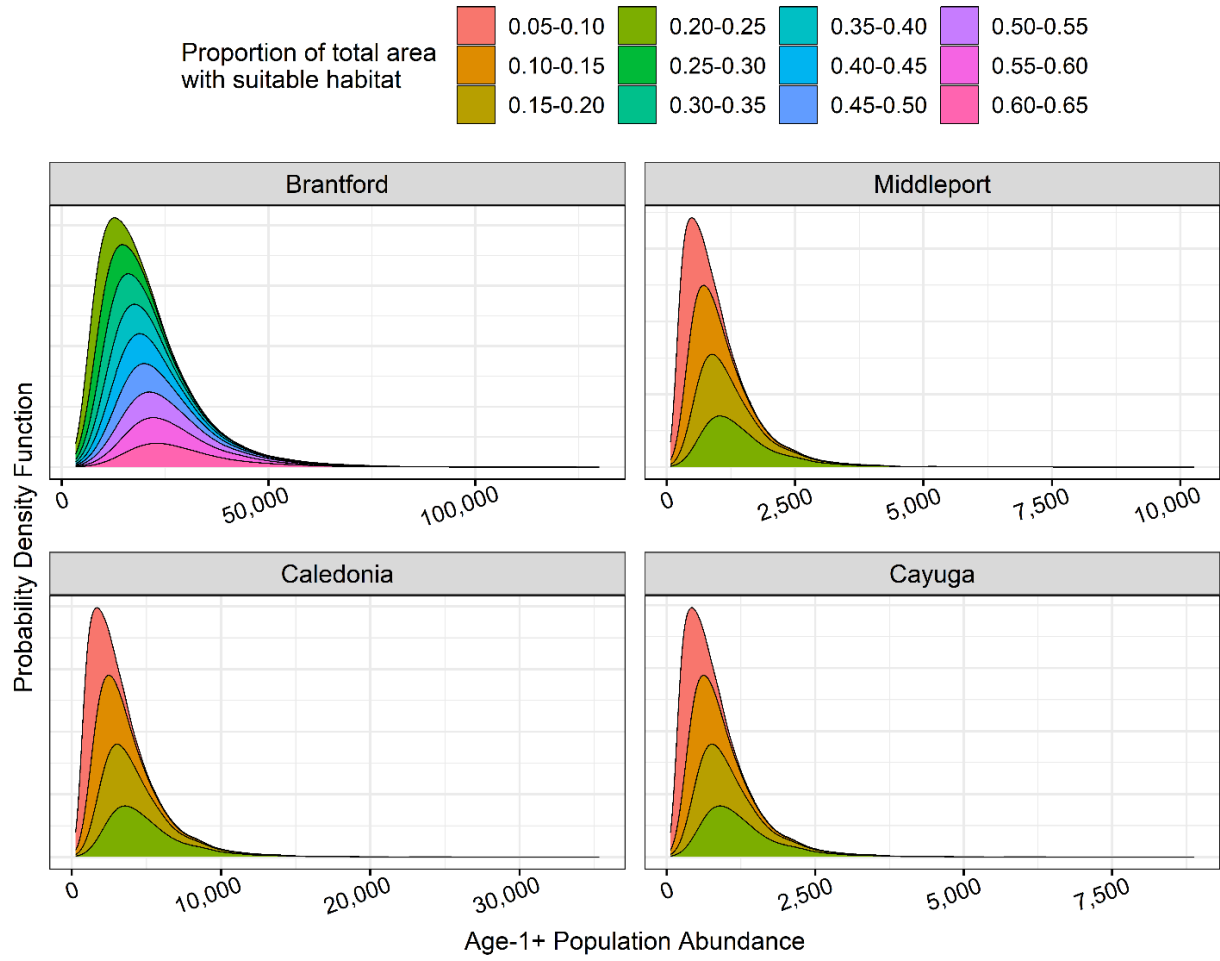


Figure 8. Indices of age-1+ Eastern Sand Darter population abundance in wadable areas of the Brantford, Middleport, Caledonia, and Cayuga sections of the Grand River based on the proportion of total area with suitable habitat (i.e., areas with at least 50% sand and fine gravel substrate; colours) and model-generated densities (Figure 7). Note the different scales of the x-axis.

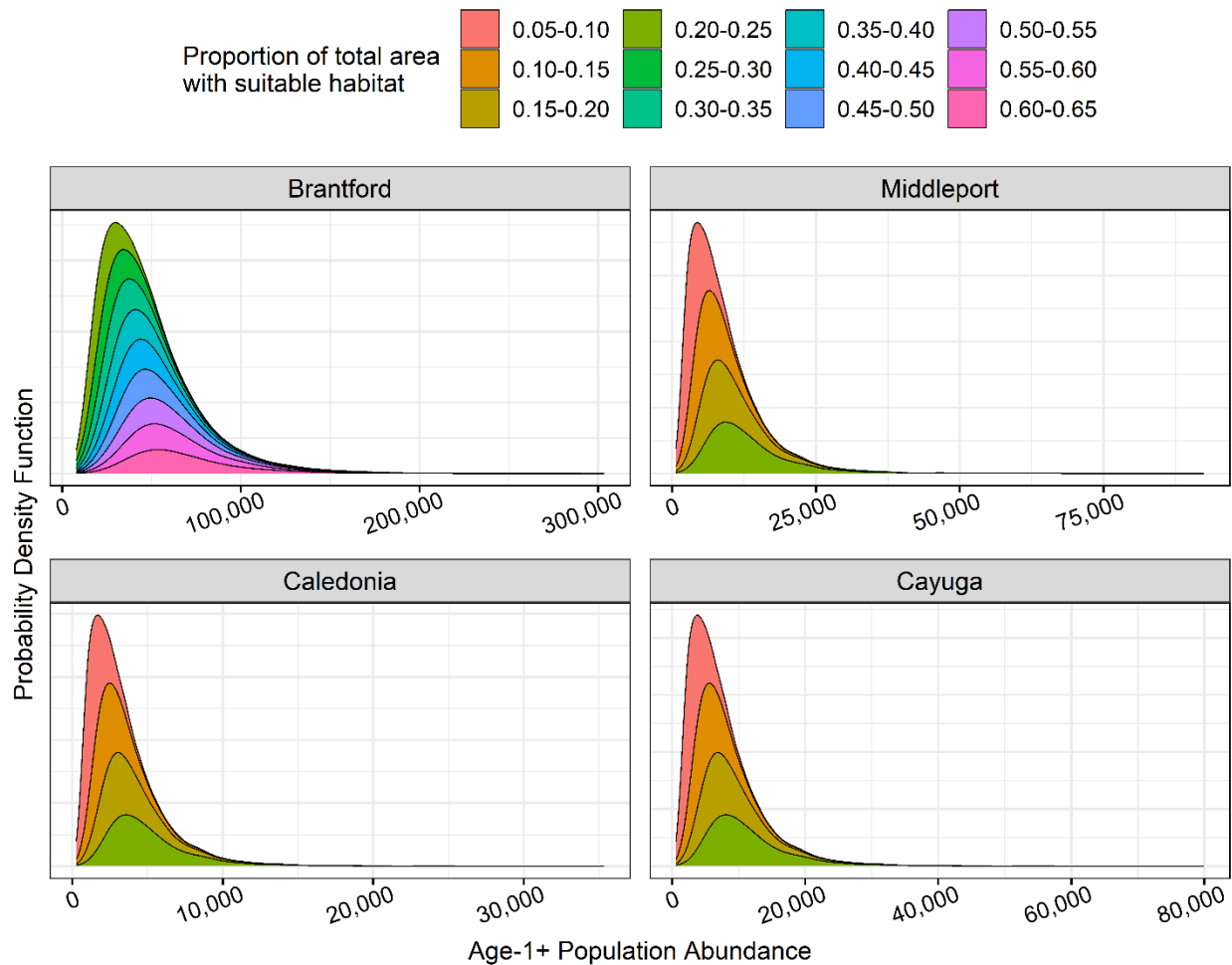


Figure 9. Indices of age-1+ Eastern Sand Darter population abundance in non-wadable areas of the Brantford, Middleport, Caledonia, and Cayuga sections of the Grand River based on the proportion of total area with suitable habitat (i.e., areas with at least 50% sand and fine gravel substrate; colours) and model-generated densities (Figure 7). Note the different scales of the x-axis.

Age-1+ population abundance was dependent on model-generated density estimates and the proportion of suitable habitat, and differed between wadable and non-wadable areas (Table 6; Figure 8, Figure 9). Median age-1+ population abundance in the Brantford section ranged between 9,438–26,051 in wadable habitat (Figure 8) and 22,021–60,798 in non-wadable habitats (Figure 9; Table 6). Abundance downstream of the Brantford section was significantly lower given the lower density estimates and proportion of suitable habitat used for generating the abundance values. Median abundance ranged between 412–4,357 age-1+ individuals in wadable areas of the Middleport, Caledonia, and Cayuga sections (Table 6). Given that density estimates from the Caledonia section were used for estimating abundance in the Middleport section, and 90% of the habitat was considered non-wadable, the greatest number of assumptions existed for abundance in the Middleport section.

Table 6. Median and mean indices of abundance for age-1+ Eastern Sand Darter in wadable, non-wadable, and combined (total; bold) habitats in the Grand River by river section and the proportion of suitable habitat ( $\geq 50\%$  sand and fine gravel). Minimum totals represent total abundance when the proportion of suitable habitat is at the minimum values considered per section and maximum totals represent total abundance when the proportion of suitable habitat is at the maximum values considered.

Section	Proportion of suitable habitat	Wadable (median)	Wadable (mean)	Non-wadable (median)	Non-wadable (mean)	Total (median)	Total (mean)
Brantford	0.20–0.25	9,438	10,637	22,021	24,820	<b>31,459</b>	<b>35,457</b>
Brantford	0.25–0.30	11,679	13,162	27,251	30,710	<b>38,930</b>	<b>43,872</b>
Brantford	0.30–0.35	13,832	15,399	32,276	35,932	<b>46,108</b>	<b>51,331</b>
Brantford	0.35–0.40	15,874	17,957	37,040	41,901	<b>52,914</b>	<b>59,858</b>
Brantford	0.40–0.45	18,028	20,172	42,066	47,067	<b>60,094</b>	<b>67,239</b>
Brantford	0.45–0.50	20,053	22,554	46,791	52,626	<b>66,844</b>	<b>75,180</b>
Brantford	0.50–0.55	22,402	25,297	52,271	59,026	<b>74,673</b>	<b>84,323</b>
Brantford	0.55–0.60	24,373	27,244	56,870	63,569	<b>81,243</b>	<b>90,813</b>
Brantford	0.60–0.65	26,051	29,370	60,786	68,531	<b>86,837</b>	<b>97,901</b>
Middleport	0.05–0.10	412	492	3,704	4,426	<b>4,116</b>	<b>4,918</b>
Middleport	0.10–0.15	695	816	6,257	7,343	<b>6,952</b>	<b>8,159</b>
Middleport	0.15–0.20	969	1,132	8,722	10,189	<b>9,691</b>	<b>11,321</b>
Middleport	0.20–0.25	1,266	1,488	11,395	13,393	<b>12,661</b>	<b>14,881</b>
Caledonia	0.05–0.10	1,416	1,692	1,416	1,692	<b>2,832</b>	<b>3,384</b>
Caledonia	0.10–0.15	2,392	2,808	2,392	2,808	<b>4,784</b>	<b>5,616</b>
Caledonia	0.15–0.20	3,335	3,896	3,335	3,896	<b>6,670</b>	<b>7,792</b>
Caledonia	0.20–0.25	4,357	5,121	4,357	5,121	<b>8,714</b>	<b>10,242</b>
Cayuga	0.05–0.10	356	425	3,202	3,827	<b>3,558</b>	<b>4,252</b>
Cayuga	0.10–0.15	601	705	5,410	6,349	<b>6,011</b>	<b>7,054</b>
Cayuga	0.15–0.20	838	979	7,541	8,810	<b>8,379</b>	<b>9,789</b>
Cayuga	0.20–0.25	1,095	1,287	9,852	11,580	<b>10,947</b>	<b>12,867</b>
<b>Total (minimum)</b>	-	<b>11,622</b>	<b>13,246</b>	<b>30,043</b>	<b>34,765</b>	<b>41,965</b>	<b>48,011</b>
<b>Total (maximum)</b>	-	<b>32,769</b>	<b>37,266</b>	<b>86,390</b>	<b>98,625</b>	<b>119,159</b>	<b>135,891</b>

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## Thames River population abundance

Recent field data were unavailable for estimating abundance of Eastern Sand Darter in the Thames River. Models to generate an index of age-1+ abundance in the Thames River were therefore built using data collected in 2006 by Dextrase (2013). Sampling design and site selection differed from the 2022 Grand River survey. The Thames River was first divided into 14 river valley segments and the number of sampled sites per segment was proportional to the length of each segment. Each river-valley segment was further divided into reaches, with sampling sites occurring among randomly selected reaches. Within the randomly selected reaches, fish community and habitat sampling were performed at up to three sites on each wadable (i.e., < 1.2 m) erosional and depositional bar (131 survey sites). A 9.2 m bag seine was used for sampling, with wing dimensions of 1.8 m x 3.7 m (6.4 mm mesh) and bag dimensions of 1.8 m x 1.8 m x 1.8 m (3.2 mm mesh; Dextrase 2013). Similar to sampling in the Grand River, a depletion design was used where individuals were removed after each seine haul and placed in separate aquaria for processing, releasing them only after all hauls had been completed.

### Thames River *N*-mixture model methods

A similar structure to the Grand River model was used when developing the Thames River *N*-mixture model given a similar approach to sampling Eastern Sand Darter:

$$C_{ij}|N_i \sim \text{Multinomial}(N_i, \pi(p_{ij})) \quad \text{Equation (8)}$$

$$N_i \sim \text{NB}(\mu_i, k) \quad \text{Equation (9)}$$

$$\text{logit}(p_{ij}) = \alpha_0 + a_1 \text{Cover}_i + u_{ij} \quad \text{Equation (10)}$$

$$\log(\lambda_i) = \log(\text{Area}_i) + \beta_0 + \beta_1 \text{SFG}_i + \beta_2 \text{Clarity}_i. \quad \text{Equation (11)}$$

The detection model covariates differed from the Grand River model because of differences between the systems and data availability. The proportion of a site estimated to consist of woody debris and boulders (i.e., cover) was used as a detection covariate, which is consistent with previous studies that model abundance in the Thames River (Dextrase 2013). Similar abundance covariates to the Grand River model were included in the model for the Thames River; specifically, the percent of sand and fine gravel substrate ( $\text{SFG}_i$ ) and water clarity ( $\text{Clarity}_i$ ; cm) as abundance covariates (Dextrase 2013). To account for differences in sampling effort (i.e., area sampled;  $\text{m}^2$ ),  $\log(\text{Area}_i)$  was included as an offset in the Thames River abundance model. This offset was unnecessary in the Grand River model as sampling effort was constant across sites.

### Thames River *N*-mixture model results

Mean detection probability was lower in the Thames River (0.141; 95% CI: 0.071–0.247) than the Grand River surveys (0.520; 95% CI: 0.354–0.668). Detection probability decreased sharply as the amount of in-stream cover increased (Table 7; Figure 10). Similar to the Grand River model, detection probability was variable across seine hauls and, therefore, a seine-specific random effect was identified as important for improving model fit (Table 7).

Table 7. Summary of N-mixture model results for the Thames River, including the mean estimate with standard deviation (SD) and the lower and upper 95% credible interval (LCI–UCI).

Sub-model	Parameters	Symbol	Mean	SD	LCI	UCI
Detection	Intercept	$\alpha_0$	-1.81	0.37	-2.57	-1.12
Detection	% Cover	$\alpha_1$	-2.02	0.67	-3.38	0.78
Detection	Dispersion parameter	$\sigma_{sample}$	0.85	0.15	0.58	1.18
Abundance	Intercept	$\beta_0$	-3.23	0.24	-3.66	-2.72
Abundance	% Sand and fine gravel	$\beta_1$	1.13	0.21	0.73	1.54
Abundance	Water clarity	$\beta_2$	0.99	0.16	0.69	1.32
Abundance	Dispersion parameter	$k$	0.62	0.13	0.41	0.90

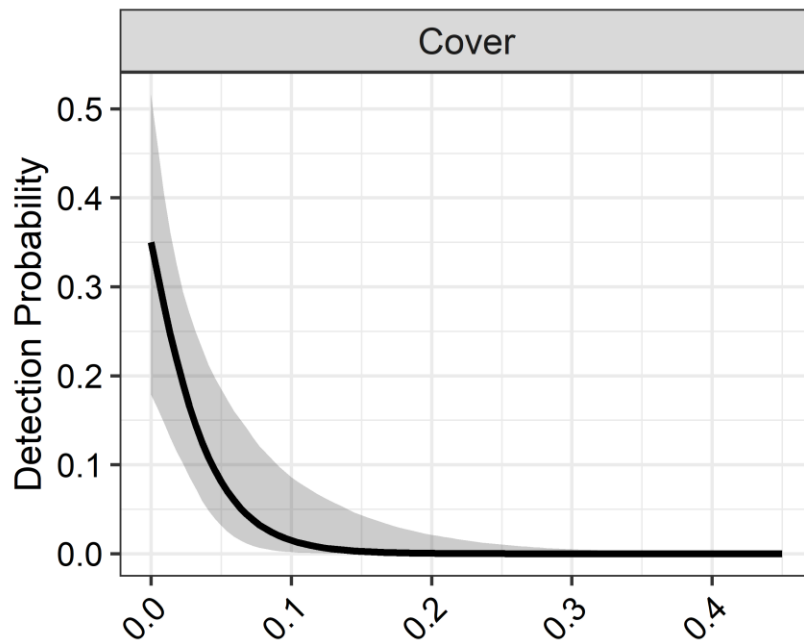


Figure 10. N-mixture model predictions of Eastern Sand Darter detection probability in the Thames River as a function of the proportion of a site consisting of woody debris and boulders (i.e., cover; x-axis). The solid line represents the median prediction and the grey area represents the 95% credible interval.

Estimated mean site density for Eastern Sand Darter in the Thames River was 0.040 fish/m<sup>2</sup> (95% CI: 0.026–0.066) and increased with water clarity and when sites had substrate with higher proportions of sand and fine gravel (Figure 11). Site-level density estimates were made for the Thames River based on average site characteristics (Figure 11), which differed from the Grand River; mean percent sand or fine gravel in the Thames River was 45.6% (range: 0–100%) and mean water clarity was 26.7 cm (range: 2.5–94.0). Similar to the Grand River model, there was no indication of lack of fit in the detection or abundance sub-models and an appropriate number of 0s was predicted from the model fit relative to what was observed (Table 4).

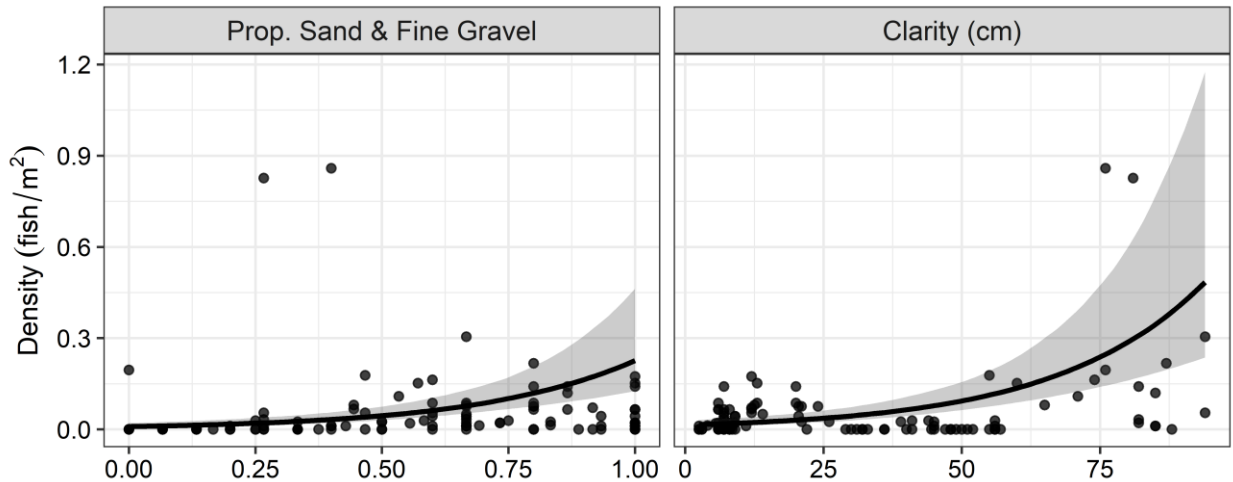


Figure 11. N-mixture model predictions of Eastern Sand Darter density (fish/m<sup>2</sup>) in the Thames River across proportion (prop.) of sand and fine gravel and water clarity (cm) measurements. Model built using data from Dextrase (2013). The solid lines represent the median predictions and the grey areas represent 95% credible intervals. Predicted densities in the proportion of sand and gravel panel incorporate mean clarity (26.7 cm) and predicted densities in the clarity panel incorporate mean proportion of sand and fine gravel (0.456).

### Thames River population abundance

Estimates of age-1+ population abundance were made for wadable and non-wadable areas across the entire area containing critical habitat of Eastern Sand Darter in the Thames River, which is approximately 5,890,400 m<sup>2</sup> (length = 148,000 m, average width = 39.8 m). A resampling 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), which was assumed to be between 5% and 50% of the total available habitat. Moreover, 50% of the total habitat was considered to be wadable based on acoustic doppler current profiler data collected in the Thames River between Komoka and Kent Bridge (Illes et al. 2025). Similar to the Grand River, the resampling procedure was repeated 20,000 times to generate a distribution of abundance estimates for Eastern Sand Darter in the area containing critical habitat within the Thames River (Figure 12).

Mean and median age-1+ abundance in the Thames River were equivalent between wadable and non-wadable habitats given that 50% of the habitat was considered wadable (Table 8). Median estimates of age-1+ abundance ranged between 8,515–54,372 in wadable habitats depending on the assumed amount of suitable habitat (Table 8). Median abundance across wadable and non-wadable habitats ranged between 17,030–108,744 age-1+ individuals.

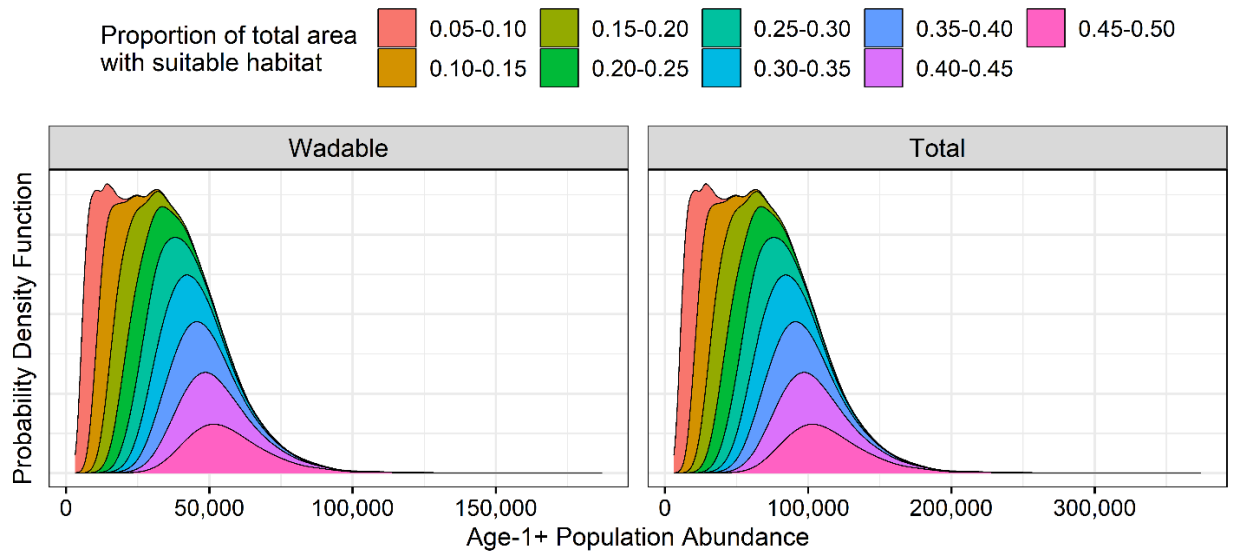


Figure 12. Indices of age-1+ abundance of the Thames River Eastern Sand Darter population in wadable sections and overall (Total) based on the proportion of the total area with suitable habitat (i.e., areas with at least 50% sand and fine gravel substrate; colours) and model-generated densities (Figure 7).

Table 8. Median and mean indices of abundance for age-1+ Eastern Sand Darter in wadable, non-wadable, and combined (total; bold) habitats in the Thames River and the proportion of total area with suitable habitat (i.e., areas with at least 50% sand and fine gravel substrate).

Proportion of suitable habitat	Wadable (median)	Wadable (mean)	Non-Wadable (median)	Non-wadable (mean)	Total (median)	Total (mean)
0.05-0.10	8,515	8,988	8,515	8,988	<b>17,030</b>	<b>17,976</b>
0.10-0.15	14,229	14,957	14,229	14,957	<b>28,458</b>	<b>29,914</b>
0.15-0.20	20,164	21,195	20,164	21,195	<b>40,329</b>	<b>42,390</b>
0.20-0.25	25,778	27,126	25,778	27,126	<b>51,555</b>	<b>54,251</b>
0.25-0.30	31,742	33,058	31,742	33,058	<b>63,485</b>	<b>66,116</b>
0.30-0.35	37,534	39,116	37,534	39,116	<b>75,068</b>	<b>78,232</b>
0.35-0.40	43,063	44,937	43,063	44,937	<b>86,125</b>	<b>89,874</b>
0.40-0.45	48,768	50,815	48,768	50,815	<b>97,537</b>	<b>101,630</b>
0.45-0.50	54,372	56,882	54,372	56,882	<b>108,744</b>	<b>113,765</b>

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

In addition to developing abundance estimates for each candidate source population, the statistical resampling approach described above was used to estimate the potential abundance of Eastern Sand Darter that could theoretically be achieved in candidate recipient locations based on the availability of suitable habitat, and assuming

1. similar densities between wadable and non-wadable areas, and
2. similar densities to the source populations.

Estimates of potential abundance could be used to determine if suitable habitat is limiting at recipient locations, or when setting long-term abundance goals for a reintroduced population.

The Ausable River has undergone significant flow modifications over the last two centuries. Habitat within and downstream of the channelized section connecting Thedford Marsh and the Village of Port Franks (i.e., 'The Cut') is considered unsuitable for Eastern Sand Darter and not included as potential habitat for the species (Figure 13). As such, potential Eastern Sand Darter habitat in the Ausable River is expected to occur upstream of The Cut to the Exeter dam (Figure 13). This section of river was divided into two subsections: upstream of The Cut to Highway 7 at Ailsa Craig (middle Section) and upstream of Highway 7 to the Exeter dam (upper section; Figure 13; Dextrase 2013). The middle section is approximately 77,689 m with an average width of  $21.98 \text{ m} \pm 10.29 \text{ SD}$  (standard deviation; data from  $n = 68$  sites), or approximately  $1,707,604 \text{ m}^2$ . The upper section is approximately 49,089 m long with an average stream width of  $12.09 \text{ m} \pm 5.06 \text{ SD}$  ( $n = 33$  sites), or approximately  $593,486 \text{ m}^2$ .

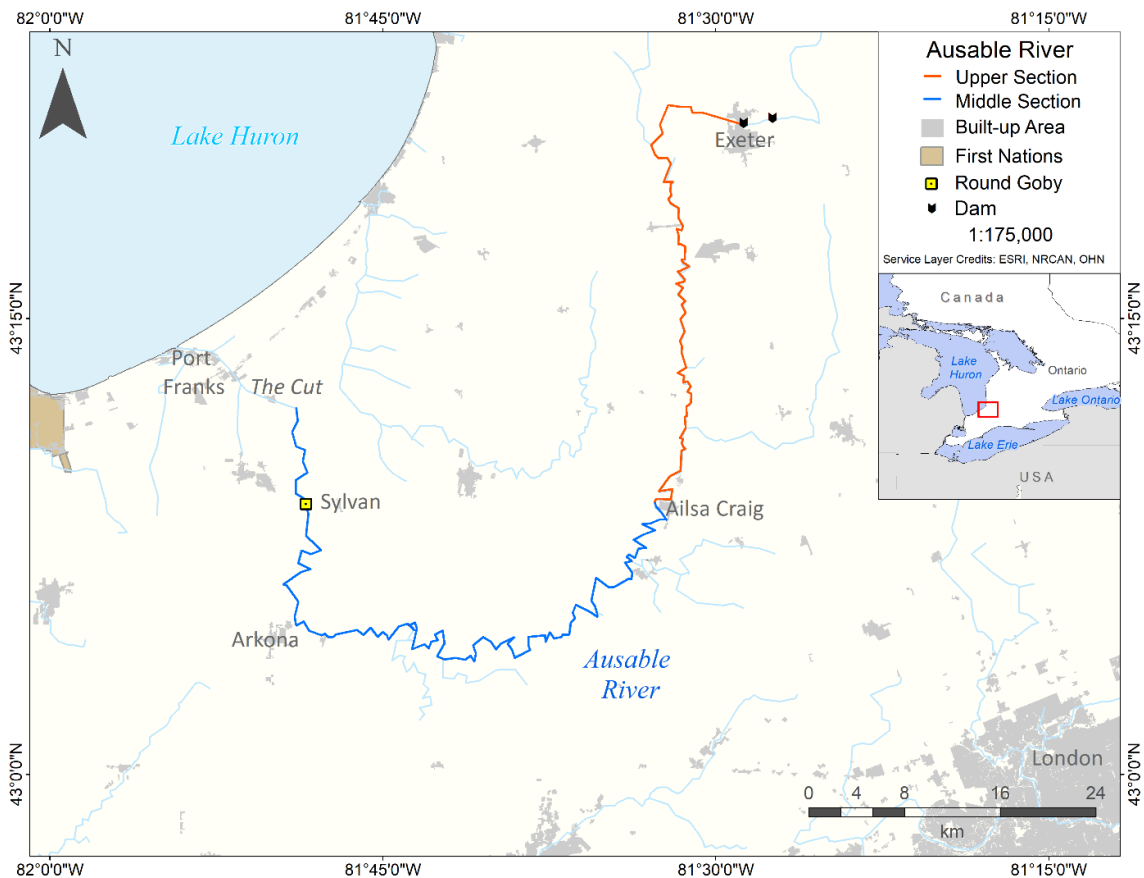


Figure 13. The division of sections in the Ausable River. Colours indicate the upstream (red) and middle river sections (blue). Farthest upstream detection record (2017) of Round Goby (*Neogobius melanostomus*) indicated with yellow square. Built-up area in grey and First Nation areas in tan.

Based on the resampling procedure where suitable (*potentially* occupied) habitat was assumed to be between 5% and 50% of total habitat, and density estimates from the Brantford and Caledonia sections of the Grand River and Thames River, mean potential population abundance across the two segments in the Ausable River was 30,488 age-1+ individuals (median = 22,499; Figure 14). 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 age-1+ individuals when suitable habitat was between 45–50% of the total habitat (Table 9; Figure 14).

Potential Eastern Sand Darter habitat in Big Otter Creek is expected to occur upstream of Vienna to Otterville, Ontario. This section of river is approximately 73,709 m long with an average width of 15.39 m  $\pm$  3.14 SD ( $n = 53$  sites), or approximately 1,134,382 m<sup>2</sup>. Mean potential abundance in Big Otter Creek was estimated as 14,994 age-1+ individuals (median = 11,027) across habitat suitability estimates (Figure 14). At the lowest level of suitable habitat (i.e., 5–10% of total habitat), median potential abundance was 3,341 age-1+ individuals while median potential abundance was 21,289 age-1+ individuals when suitable habitat was between 45–50% of the total habitat (Table 9; Figure 14).

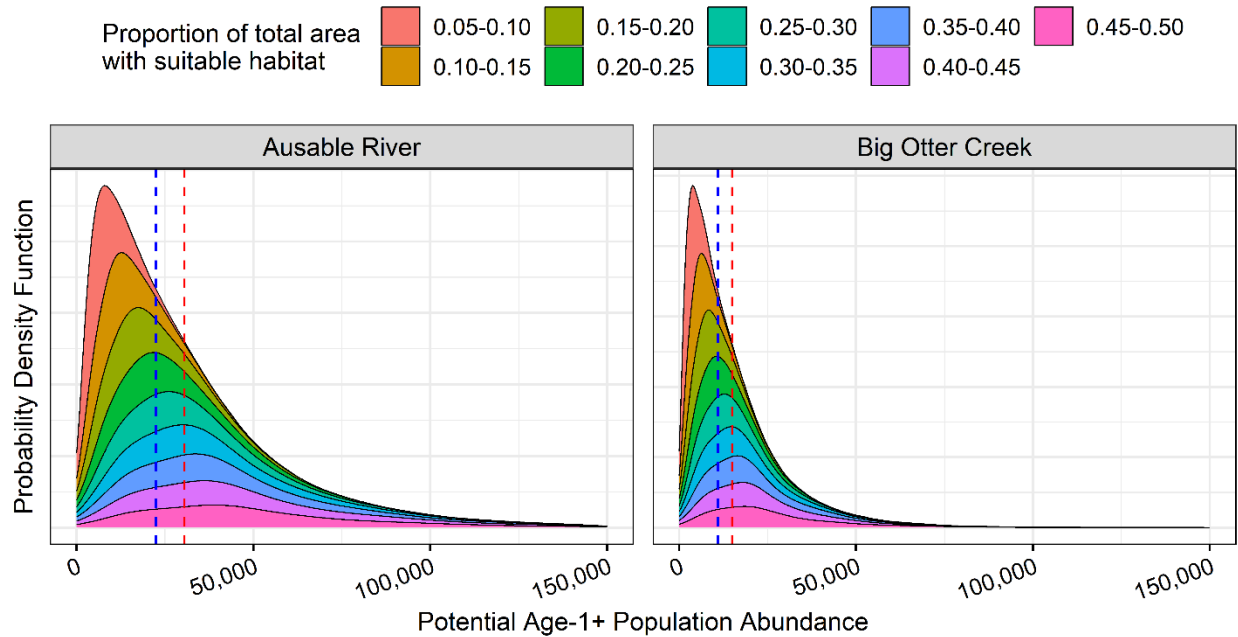


Figure 14. Potential age-1+ abundance of a reintroduced Eastern Sand Darter population in the Ausable River and Big Otter Creek based on the proportion of total area with suitable habitat (i.e., areas with at least 50% sand and fine gravel substrate; colours) and model-generated density estimates from the Brantford and Caledonia sections of the Grand River, and the Thames River. Dashed lines indicate median (blue) and mean (red) potential population abundances.

Table 9. Median potential abundance of age-1+ Eastern Sand Darter in the Ausable River and Big Otter Creek based on the density estimates from the Grand and Thames rivers and the proportion of total area with suitable habitat (i.e., areas with at least 50% sand and fine gravel substrate).

Waterbody	Proportion of total area with suitable habitat	Median abundance
Ausable River	0.05–0.10	6,766
Ausable River	0.10–0.15	11,270
Ausable River	0.15–0.20	15,874
Ausable River	0.20–0.25	20,476
Ausable River	0.25–0.30	25,206
Ausable River	0.30–0.35	29,551
Ausable River	0.35–0.40	34,449
Ausable River	0.40–0.45	38,138
Ausable River	0.45–0.50	43,221
Big Otter Creek	0.05–0.10	3,341
Big Otter Creek	0.10–0.15	5,606

Waterbody	Proportion of total area with suitable habitat	Median abundance
Big Otter Creek	0.15–0.20	7,821
Big Otter Creek	0.20–0.25	10,143
Big Otter Creek	0.25–0.30	12,341
Big Otter Creek	0.30–0.35	14,634
Big Otter Creek	0.35–0.40	16,724
Big Otter Creek	0.40–0.45	19,072
Big Otter Creek	0.45–0.50	21,289

### 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 are similar to those presented in Lamothe et al. (2021). First, 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). These types of models use population vital rates to project age-specific population abundances over time, where population growth rate ( $\lambda$ ; see Table 10 for definitions of symbols) represents the long-term projection of population status based on current or simulated conditions. When  $\lambda = 1$ , the population is stable; when  $\lambda > 1$ , the population is growing exponentially; and when  $\lambda < 1$ , the population is declining.

Table 10. Parameter values incorporated in the matrix population model for Eastern Sand Darter. Fixed parameters were held constant across model runs and uncertain parameters were sampled randomly from the specified distribution.

Parameter	Definition	Value
$T_{max}$	Longevity	<i>Unif</i> (3, 4)
$T_{mat}$	Age-at-maturity	<i>Unif</i> (1, 2)
$S_{1+}$	Age-1+ survival rate	<i>Beta</i> (15.88, 25.26)
$S_0$	Age-0 survival rate	Solved for
$CV_S$	Survival rate coefficient of variation	0.2
$f$	Clutch size	<i>Gamma</i> (55.7, 0.91)
$c$	Clutch number per year	<i>Unif</i> (1, 2, 3)
$CV_f$	Clutch size coefficient of variation	<i>Unif</i> (0.05, 0.15)
$\phi$	Sex ratio at birth	0.5
$\rho$	Proportion mature at age	0.91, 1, 1, 1
$Pr_{cat}$	Probability of a catastrophic event	<i>Unif</i> (0.05, 0.20)
$M$	Translocation mortality	<i>Unif</i> (0.20, 0.80)
$\lambda_{max}$	Maximum population growth rate	<i>Unif</i> (1.56, 2.69)
$n_R$	Number of removals	<i>Unif</i> (1, 1000)
$Y_R$	Years of removals	1–10
$n_A$	Number of additions	<i>Unif</i> (1, 1000)

Parameter	Definition	Value
$Y_A$	Years of additions	1–10

### Parameterization

Model parameters and outputs are specific to female Eastern Sand Darter. Many aspects of Eastern Sand Darter life history are uncertain or variable among populations (Finch et al. 2018). Rather than developing matrix population models for differing life-history strategies (*sensu* Lamothe et al. 2021), uncertainty in life-history parameters was incorporated into a single model by randomly selecting the uncertain parameter from a specified probability distribution allowing these variables to vary among simulation replicates and provide a range of plausible outputs (Table 10). This approach allows different model runs to incorporate different parameter combinations and provides results that span the full extent of the uncertainty. Variables incorporated into population models as distributions included longevity ( $T_{max}$ ), age-at-maturity ( $T_{mat}$ ), age-1 survival rate ( $S_{1+}$ ), clutch size ( $f$ ), clutch number per year ( $c$ ), clutch size coefficient of variation ( $CV_f$ ), and maximum population growth rate ( $\lambda_{max}$ ; Table 10).

Longevity ( $T_{max}$ ) and age-at-maturity ( $T_{mat}$ ) are important parameters for defining the structure of the population model. In Canada, longevity may be age-3 (Finch et al. 2013) or age-4 (Drake et al. 2008) and  $T_{mat}$  has been observed as age-1 or age-2 across populations (Finch et al. 2013). To allow for variability in  $T_{max}$  and  $T_{mat}$  among simulations the matrix was structured as a stage-structured matrix with three stages (Figure 15): stage 1 represents age-1 Eastern Sand Darter, stage 2 represents age-2 Eastern Sand Darter, and stage 3 represents age-3+ Eastern Sand Darter.

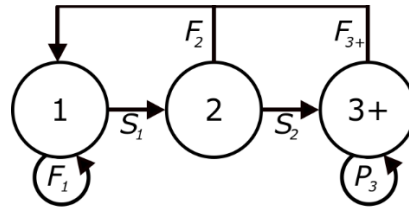


Figure 15. Generalized life cycle used to model Eastern Sand Darter population dynamics.  $F_i$  represents stage-specific annual fertility,  $S_i$  represents stage-specific survival rate, and  $P_3$  represents the probability of surviving and remaining in stage 3. The projection matrix  $\mathbf{A}$  is

$$\mathbf{A} = \begin{bmatrix} F_1 & F_2 & F_3 \\ S_1 & 0 & 0 \\ 0 & S_2 & P_3 \end{bmatrix}. \quad \text{Equation (12)}$$

Stage-specific abundance ( $\mathbf{n}$ ) each year ( $y$ ) is calculated from:

$$\mathbf{n}_{y+1} = \mathbf{A}_{n,y} \mathbf{n}_y, \quad \text{Equation (13)}$$

where  $\mathbf{n}$  is a vector of stage-specific abundance. The population projection matrix ( $\mathbf{A}$ ) varies among years due to environmental conditions and population size impacting vital rates.

Stage-based matrix models incorporate estimates of stage-specific fertility ( $F_i$ ), stage-specific survival rate ( $S_i$ ), and the probability of survival and remaining in stage 3 ( $P_3$ ). Fertility ( $F_i$ ) represents the number of female offspring produced per adult female annually and comprises all reproductive parameters including clutch size, the number of clutches produced per year, sex ratio, spawning periodicity, and survival from the egg state to age-1. The probability of survival and remaining in stage 3 ( $P_3$ ) is a function of stage-3 survival and transition probability ( $\tau_3$ ) describing the likelihood of remaining in stage 3.  $P_3$  is calculated as:

$$P_3 = S_3(1 - \tau_3) \quad \text{Equation (14)}$$

with (Caswell 2001):

$$\tau_3 = \frac{S_3^{T_{max}-2} - S_3^{(T_{max}-2-1)}}{(S_3^{T_{max}-2} - 1)}. \quad \text{Equation (15)}$$

When  $T_{max}$  is 4,  $P_3$  describes the number of age-3 Eastern Sand Darter that survive to age-4 and when  $T_{max}$  is 3,  $P_3$  reduces to 0 such that no individuals survive to age-4.

Survival rate was estimated for Eastern Sand Darter populations using a Chapman-Robson catch curve analysis (Finch et al. 2018). Mean survival rate was 0.386 (SD = 0.075) for age-1+ individuals. In the model, annual survival was assumed to be the same for age-1+ age-classes and was included as a stochastic variable. Age-1+ survival was drawn from a beta distribution such that the value could not be less than 0 or greater than 1 with shape parameters 15.88 and 25.26, which gives a mean of 0.386 (SD = 0.075). Age-0 survival rate is unknown and was solved for to give a projection matrix with a particular population growth rate. Survival rate was varied inter-annually to represent environmental stochasticity. Finch et al. (2018) estimated that annual survival rate varied among years with a coefficient of variation (CV) of 0.2.

Fertility describes the number of female offspring surviving to age-1 produced annually per adult female in the population and is calculated as:

$$F_i = \varphi \rho_i f c a_f d S_0. \quad \text{Equation (16)}$$

$\varphi$  is the sex ratio at birth, which was assumed to be 50%, and  $\rho_i$  is the proportion of females mature at age- $i$ . Finch et al. (2018) observed that within a population that matured at age-1, approximately 91% of females were mature at age-1 and all individuals age-2+ were mature; therefore,  $\rho_1$  was set to 0.91 when  $T_{mat}$  was 1 and 0 when  $T_{mat}$  was 2, and  $\rho_2$  and  $\rho_3$  were set to 1 (Table 10). Fecundity ( $f$ ), also described as average clutch size, is the number of eggs produced per clutch per female. Reported mean clutch sizes for Eastern Sand Darter range from 56 to 71.5 (COSEWIC 2009, 2022, Finch et al. 2013). As such, clutch size was incorporated as a stochastic parameter with a mean of 61.2 (SD = 8.2; Faber 2006, Finch et al. 2013). The value for clutch size was drawn from a gamma distribution such that clutch size was always positive with shape and rate parameters of 55.70 and 0.91. This distribution results in 99% confidence intervals of 48.4–84.4, which was deemed a reasonable amount of variation. The number of clutches produced per year ( $c$ ) was allowed to vary from 1 to 3 between simulation replicates. Clutch size was varied across years to simulate environmental stochasticity. The amount that clutch size may vary is unknown; as a result, the CV of  $f$  was incorporated as a stochastic variable and drawn from a uniform distribution between 0.05 and 0.20. The lower limit (0.05) was selected to represent low inter-annual variability and the upper limit (0.20) was selected to mirror the variability of survival rate.  $S_0$  is age-0 survival rate (i.e., egg to age-1).

Density dependence ( $d$ ) was assumed to act during the first year of life and implemented using a Beverton-Holt function with age-0 survival rate a function of adult density:

$$d_y = \frac{S_{0,max}/S_{0,1}}{1 + b/KN_y} \quad \text{Equation (17)}$$

where  $N$  is age-1+ density,  $K$  is carrying capacity of age-1 females,  $b$  is the density-dependence coefficient,  $S_{0,1}$  is age-0 survival rate when the population is stable, and  $S_{0,max}$  is age-0 survival when the population is at maximum population growth rate ( $\lambda_{max}$ ). In a previous population model (Lamothe et al. 2021),  $\lambda_{max}$  was estimated from an allometric relationship (Randall et al. 1995) and was assumed to vary between 1.56 and 2.69. In this model  $\lambda_{max}$  was incorporated as

a stochastic variable and drawn from a uniform distribution with a minimum value of 1.56 and maximum value of 2.69 [i.e.,  $Unif(1.56, 2.69)$ ; Table 10].  $S_{0,max}$  was then solved for in the same manner as  $S_{0,1}$  to find the value that resulted in a population growing at its maximum rate. The value  $b$  was calculated by rearranging Equation 7 with  $N = K$ , as:

$$b = S_{0,max} / S_{0,1} - 1. \quad \text{Equation (18)}$$

Allee effects ( $a_f$ ), which represent a reduction in population growth rate when populations are small, was modelled as a proportional reduction in fertility rate shaped as a Holling type III function:

$$a_{f,y} = \frac{N_y^2}{a^2 + N_y^2} \quad \text{Equation (19)}$$

where  $N_t$  is age-1 abundance in year  $y$  and  $a$  is the Allee effect coefficient representing the age-1+ population size at 50% reproductive success (Lamothe et al. 2021). The value of  $a$  was fixed at 50 across simulations.

The variation in vital rates that results from small population size is termed demographic stochasticity (Lande 1993, Morris and Doak 2002). Demographic stochasticity was assumed to impact age-1+ survival rate and clutch size when age-1+ abundance  $< 500$ . Age-1+ survival rate at low abundance was a function of a binomial distribution with size of  $N_{1+}$  and probability of  $S_t$ , where:

$$S_N = \text{Binomial}(N, S_y) / N. \quad \text{Equation (20)}$$

Clutch size at low abundance was assumed to follow a Poisson distribution with mean  $N_{1+}$ , where

$$f_N = \text{Poisson}(N). \quad \text{Equation (21)}$$

### Minimum viable population simulation

Minimum viable population size ( $MVP$ ) describes the absolute minimum population size of age-1+ individuals that has a certain probability of remaining extant over some period of time despite the continuous effects of stochasticity and catastrophic events (Shaffer 1981). Here,  $MVP$  was defined as the absolute minimum population size of age-1+ individuals that has a 99% probability of remaining extant over the next 100 years [approximately 52.7 Eastern Sand Darter generations, where generation time ( $\zeta$ ) was estimated from stochastic projection matrices (Caswell 2001) with  $\lambda = 1$  where  $\zeta = 1.90$  (95% CI: 1.35–2.62)]. Simulation analyses that incorporated parameter uncertainty, environmental stochasticity, Allee effects, demographic stochasticity, and density dependence were used to estimate  $MVP_{99\%}$ . The mean and variance of  $MVP_{99\%}$  were used as criteria for evaluating 1) the risk to source populations when removing individuals and 2) the successful establishment of Eastern Sand Darter in the recipient location.

The estimated  $MVP$  of small-bodied freshwater fishes can be strongly impacted by events that cause a decrease in population size greater than 50% (i.e., catastrophic event; Reed et al. 2003a,b, Vélez-Espino and Koops 2012). Meta-analyses have determined that catastrophic events among vertebrate populations, evaluated as a one-year decrease in population size  $\geq 50\%$ , occurred at an average rate of 14% per generation (Reed et al. 2003a); however, the actual rate of catastrophic events for Eastern Sand Darter populations is unknown. As such, the probability of catastrophe ( $Pr_{cat}$ ) was included in  $MVP$  simulations as a stochastic parameter drawn from a uniform distribution with limits of 0.05 and 0.20 (Table 10). The effects of catastrophic events were simulated to occur across all life stages simultaneously and were drawn randomly from a beta distribution scaled between 0.50 and 1.00 with shape parameters of 0.762 and 1.50 (Reed et al. 2003a;  $Beta(0.762, 1.5) \times (1 - 0.5) + 0.5$ ), representing the

probability of a 50–100% decline in population size. On average, a 5% per generation catastrophe rate has a catastrophe occurring every 38 years, while a 20% per generation catastrophe rate has a catastrophe occurring every 9.5 years.

*MVP* simulations were conducted with various initial abundances of adult females, ranging from 150 to 60,000 [initial density representing carrying capacity ( $K$ ) where  $\lambda = 1$ ]. The simulations were conducted for 100 years and replicated 10,000 times. The number of extirpations (< 1 remaining female) was tracked for populations across simulations. The probability of extirpation ( $Pr_{ext}$ ) was modelled using logistic regression, where:

$$Pr_{ext} = \frac{1}{1+e^{-(X\beta)}}. \quad \text{Equation (22)}$$

Here,  $\mathbf{X}$  is a matrix of all covariates and  $\beta$  is a vector of coefficients, including the intercept. Covariates considered in the model included  $\log_{10}$ -transformed initial female density [ $\log_{10}(Na)$ ],  $T_{mat}$ ,  $T_{max}$ ,  $\lambda_{max}$ ,  $S$ ,  $f$ ,  $c$ ,  $CV_f$ , and  $Pr_{cat}$ . Only variables with  $p < 0.05$  were included in the final model (Table 11).

Mean estimated  $MVP_{99\%}$  for Eastern Sand Darter was 16,651 age-1+ females (95% CI: 3,098–51,229; Figure 16), or approximately 33,302 age-1+ individuals (i.e., 16,651×2). Carrying capacity [ $\log_{10}(K)$ ] had the strongest influence on the probability of extirpation, followed by  $Pr_{cat}$ ,  $\lambda_{max}$ , and  $T_{mat}$  (Table 11).

*Table 11. Results of the Eastern Sand Darter minimum viable population size (MVP) logistic regression. Variables were transformed to z-scores prior to modeling.  $K$  = carrying capacity;  $Pr_{cat}$  = probability of a catastrophic event;  $\lambda_{max}$  = maximum population growth rate;  $T_{mat}$  = age-at-maturity; SE = standard error;  $p$  = significance level.*

Variable	Estimate	SE	z-statistic	p
Intercept	-4.827	0.085	-56.602	< 0.001
$\log_{10}(K)$	-1.249	0.036	-35.058	< 0.001
$Pr_{cat}$	0.879	0.054	16.136	< 0.001
$\lambda_{max}$	-0.469	0.050	-9.418	< 0.001
$T_{mat}$	-0.188	0.048	-3.938	< 0.001

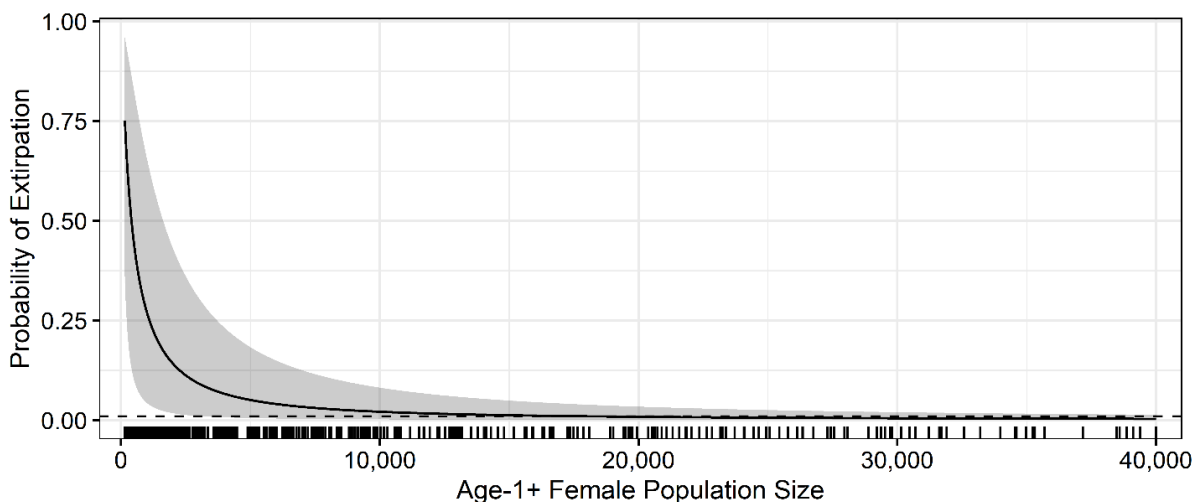


Figure 16. The probability of extirpation for Eastern Sand Darter as a function of age-1+ female population size. The rug plot indicates extirpation occurrences. The solid line represents the logistic regression mean trend with 95% confidence intervals (grey area) where the probability of a catastrophic event ranges from 5% to 20% per generation and maximum population growth rate ranges from 1.56 to 2.69. The dashed line indicates a 1% probability of extirpation.

### Effects of removals on source populations

A population viability analysis was used to quantify the impact of removing individuals from a source population. Simulations were run for 20 years using 40,000 repetitions, where the per generation probability of a catastrophic event was randomly selected for each repetition from a uniform distribution between 5% and 20% (Table 10). Initial source population size ( $Na$ ) was sampled from the model-generated abundance distribution of the Grand or Thames river populations across wadable and non-wadable habitats and halved to represent only females for each repetition. Similar to the MVP analysis, removal simulations incorporated Allee effects, demographic and environmental stochasticity, and density dependence. Annual removals were performed for 1 to 10 years, with  $n_R$  age-1 to  $t_{max}$  females removed from a source population for each repetition, where  $n_R$  ranged from 0 to 500 (Table 10). Removals of individuals from the source population took place before spawning occurred. Significant harm to the source populations was considered to occur if the source population declined below mean  $MVP_{99\%}$  (16,651 age-1+ females; Figure 16) at any point during the simulation, indicating a chance that the population could be extirpated over the next 100 years. Mortality resulting from removals was in addition to the natural mortality rate occurring in the population.

Logistic regression was used to identify influential covariates on the probability of removal impacts (i.e., 1 = falling below  $MVP_{99\%}$  = harm; 0 = no harm). Variables considered in the modelling included  $\log_{10}$ -transformed total number of removals [ $\log_{10}(R)$ ], calculated as the number of annual removals ( $n_R$ ) multiplied by the number of years removal occurred, along with  $T_{max}$ ,  $T_{mat}$ ,  $S$ ,  $f$ ,  $c$ ,  $CV_f$ ,  $\lambda_{max}$ , and  $Pr_{cat}$ . Variables with  $p < 0.05$  were included in the final model. Covariates were transformed to z-scores prior to modelling for reporting.

The two most influential variables on the probability of removal impacts were  $\log_{10}(Na)$  and  $Pr_{cat}$  (Table 12). As abundance of the source population increased, the probability of extirpation decreased (Figure 17), where an age-1+ female population size of approximately 41,042 individuals had a 50% probability of experiencing a decrease in abundance to below  $MVP_{99\%}$ . Three life-history characteristics ( $\lambda_{max}$ ,  $CV_f$ ,  $S$ ) were included in the final model but had small effect sizes (Table 12).

Table 12. Logistic regressions of the probability of removal impacts on source populations. Variables were scaled and centered prior to modeling.  $\log_{10}(Na)$  = log-10 transformed initial female density;  $Pr_{cat}$  = probability of a catastrophic event;  $CV_f$  = clutch size coefficient of variation ;  $\lambda_{max}$  = maximum population growth rate;  $S$  = age-1+ survival rate; SE = standard error;  $p$  = significance level.

Variable	Estimate	SE	z-statistic	p
Intercept	2.434	0.016	149.255	< 0.001
$\log_{10}(Na)$	-1.606	0.015	-105.784	< 0.001
$Pr_{cat}$	0.501	0.012	42.933	< 0.001
$CV_f$	0.040	0.011	3.583	< 0.001
$\lambda_{max}$	-0.035	0.011	-3.078	0.002
$S$	-0.028	0.011	-2.534	0.011

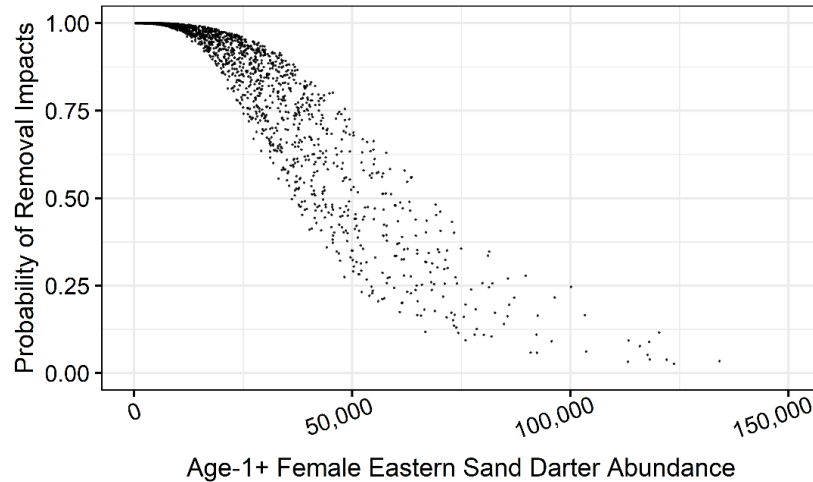


Figure 17. Probability of reducing age-1+ female Eastern Sand Darter abundance below the mean minimum viable population ( $MVP_{99\%}$ ) size (i.e., probability of removal impacts).

### Reintroduction scenarios

Simulations were used to assess the potential performance of translocated Eastern Sand Darter in recipient systems. In these simulations, the initial female-only age-1+ population size at the recipient system was set to 0 and carrying capacity ( $K$ ) was sampled from the previously described model-generated potential population abundance distributions for the Ausable River or Big Otter Creek, independently, for each repetition divided by two (i.e., females only). Here,  $K$  describes the average population abundance for age-1+ female Eastern Sand Darter in the average, modelled habitat conditions from the Grand or Thames rivers. Reintroduction efforts ranged between 1 and 500 age-1+ females per year for 1–10 years. Reintroductions took place after the spawning period, with individuals unable to spawn until the following year. This conservatively assumed that translocated individuals would not spawn in the year that they were reintroduced (but see Poly 2003). Translocation mortality ( $M$ ) was incorporated into the simulations, which represented the situation where individuals perished during the act of transport or immediately following reintroduction, or individuals survived but emigrated from the reintroduction location.  $M$  was incorporated as a random variable selected for each repetition from a uniform distribution between 20% and 80% (Table 10). Populations were monitored for 20 years regardless of the number of years that Eastern Sand Darter was reintroduced. A total of 40,000 repetitions were performed per scenario. Success was evaluated using two thresholds:

1. having an abundance greater than the lower 95% CI of Eastern Sand Darter  $MVP_{99\%}$  ( $n = 3,098$  age-1+ females) at any point following the conclusion of reintroduction efforts; and,
2. having an abundance greater than mean  $MVP_{99\%}$  ( $n = 16,651$  age-1+ females) at any point following the conclusion of reintroduction efforts.

Logistic regression was used to identify influential covariates on reintroduction success (1 = 3,098 or 16,651 age-1+ females after 20 years; 0 = failure to reach 3,098 or 16,651 age-1+ females). Variables considered in the modelling included  $\log_{10}$ -transformed total number of additions [ $\log_{10}(A)$ ], calculated as the number of individuals reintroduced in a year multiplied by the number of years of additions, along with  $T_{max}$ ,  $T_{mat}$ ,  $S$ ,  $f$ ,  $c$ ,  $CV_f$ ,  $\lambda_{max}$ , and  $Pr_{cat}$ . Only variables with  $p < 0.05$  were included in the final model. Covariates were converted to  $z$ -scores prior to modelling.

The two most influential variables on reintroduction success were the total number of age-1+ female additions [ $\log_{10}(A)$ ] and carrying capacity [ $\log_{10}(K)$ ]; Table 13]; the probability of success increased with the number of additions (Figure 18) and carrying capacity. When the lower 95% confidence interval of  $MVP_{99\%}$  was used as the success threshold, [ $\log_{10}(A)$ ] was the most important factor whereas [ $\log_{10}(K)$ ] was the most important factor when mean  $MVP_{99\%}$  was used as the success threshold (Table 13). The probability of success improved with  $\lambda_{max}$ , while  $M$  had a negative effect on success (Table 13).

*Table 13. Logistic regressions of reintroduction success. Variables were scaled and centered prior to modeling.  $MVP_{99\%}$  LCI = Minimum viable population size (99%) lower credible interval (3,098 age-1+ females);  $\log_{10}(A)$  = log10-transformed total number of additions;  $\log_{10}(K)$  = log10-transformed carrying capacity;  $M$  = translocation mortality;  $\lambda_{max}$  = maximum population growth rate;  $T_{mat}$  = age-at-maturity;  $S$  = age-1+ survival rate;  $Pr_{cat}$  = probability of a catastrophic event.  $T_{max}$  = longevity;  $CV_f$  = clutch size coefficient of variation; SE = standard error;  $p$  = significance level.*

Threshold	Variable	Estimate	SE	z-statistic	p
$MVP_{99\%}$ LCI	Intercept	1.094	0.012	92.47	< 0.001
$MVP_{99\%}$ LCI	$\log_{10}(A)$	2.072	0.017	124.18	< 0.001
$MVP_{99\%}$ LCI	$\log_{10}(K)$	1.648	0.014	113.82	< 0.001
$MVP_{99\%}$ LCI	$M$	-0.641	0.011	-56.65	< 0.001
$MVP_{99\%}$ LCI	$\lambda_{max}$	0.512	0.011	46.28	< 0.001
$MVP_{99\%}$ LCI	$T_{mat}$	0.393	0.011	36.09	< 0.001
$MVP_{99\%}$ LCI	$S$	0.300	0.011	27.61	< 0.001
$MVP_{99\%}$ LCI	$Pr_{cat}$	-0.233	0.011	-21.71	< 0.001
Mean $MVP_{99\%}$	Intercept	-4.154	0.035	-120.051	0.000
Mean $MVP_{99\%}$	$\log_{10}(K)$	3.874	0.035	111.435	0.000
Mean $MVP_{99\%}$	$\log_{10}(A)$	1.388	0.020	68.201	0.000
Mean $MVP_{99\%}$	$\lambda_{max}$	0.736	0.015	48.265	0.000
Mean $MVP_{99\%}$	$M$	-0.410	0.014	-28.350	0.000
Mean $MVP_{99\%}$	$T_{mat}$	0.392	0.014	27.052	0.000
Mean $MVP_{99\%}$	$Pr_{cat}$	-0.283	0.014	-19.673	0.000
Mean $MVP_{99\%}$	$S$	-0.064	0.014	-4.511	0.000
Mean $MVP_{99\%}$	$T_{max}$	-0.063	0.014	-4.465	0.000
Mean $MVP_{99\%}$	$CV_f$	0.057	0.014	4.032	0.000

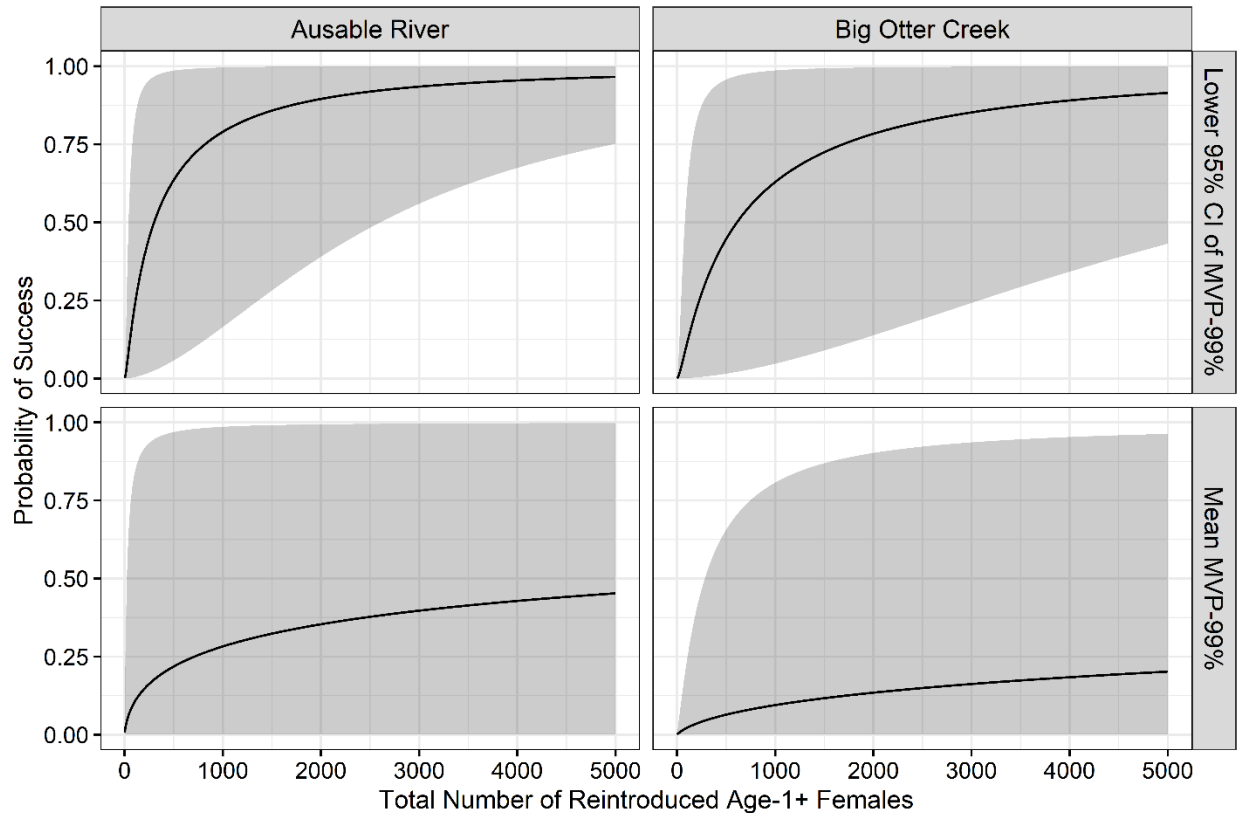


Figure 18. Probability of age-1+ female abundance in the recipient location being above the lower 95% confidence interval (CI) of estimated minimum viable population size ( $MVP_{99\%}$ ; 3,098 age-1+ females) and mean  $MVP_{99\%}$  (16,651 age-1+ females) for Eastern Sand Darter in the Ausable River and Big Otter Creek after reintroductions cease (i.e., probability of success) as a function of the total number of age-1+ females individuals reintroduced.

### Summary of population modeling results

The results presented above provide the first indices of population abundance for Eastern Sand Darter in Canada. Population abundances were based on density estimates from  $N$ -mixture models fit to data collected in 2022 (Grand River) and 2006 (Thames River) and extrapolated across the estimated area of suitable habitat and divided between wadable and non-wadable areas. Simulations were then conducted to evaluate the potential harm caused by removing individuals from these populations and the probability of establishing a population in the Ausable River or Big Otter Creek. The results of these simulations suggested that the abundance of Eastern Sand Darter in the Grand or Thames rivers could drop below mean  $MVP_{99\%}$  regardless of whether individuals were removed from the population. Nevertheless, the probability of establishing a population in the Ausable River or Big Otter Creek was high, particularly when 1,000 or more age-1+ females were reintroduced. However, careful interpretation of these results is warranted given the uncertainty across the variables included in the modelling, which is expanded upon below.

Population abundance estimates were made using estimated densities from the Brantford and Caledonia sections of the Grand River, and the Thames River. Density estimates were highest in the Brantford section of the Grand River, followed by the Thames River, and the Caledonia section of the Grand River (Figure 7). The density estimates from the Grand and Thames rivers were generated using different models on data that were collected using different approaches.

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Density estimates for the Thames River population were based on data collected from sites with a lower proportion of sand and fine gravel and reduced water clarity compared to data collected and modelled from the Grand River. Generally, there is greater confidence in the modelling results from the Grand River than the Thames River given the amount of time elapsed since the last large-scale sampling efforts. Moreover, less is known about the total amount of suitable habitat available for Eastern Sand Darter in the Thames River than the Grand River.

Density estimates were scaled up to the population level based on estimates of suitable habitat and extrapolated across wadable and non-wadable habitats. Sampling in the Grand River in 2022 and the Thames River in 2006, which the  $N$ -mixture models were constructed from, was only performed in wadable habitats and therefore the applicability of these densities to non-wadable habitat is unknown. However, it is unlikely that density estimates in the non-wadable habitats fall outside of the density distributions that were used to extrapolate population estimates (Figure 7). If the density of Eastern Sand Darter was lower in the non-wadable habitats, then abundance would be reduced compared to the presented results. The opposite outcome would occur if density in non-wadable habitats was higher than in wadable habitat.

Scaling up density estimates based on an uncertain amount of suitable habitat led to significant uncertainty in population abundance estimates (Figure 8; Figure 9; Figure 12). Nevertheless, nearly all of the simulations suggested that source population abundance in the Grand River was above mean  $MVP_{99\%}$  (19,716 of 20,000 simulations) compared to 78% for the Thames River (15,652 of 20,000). Source population size was the most important factor influencing reductions in abundance declining below mean  $MVP_{99\%}$  (Table 12). The probability of the source populations crossing the most conservative harm threshold (mean  $MVP_{99\%}$ ) was largely driven by stochastic events, such as catastrophes. The number of Eastern Sand Darter removed from the system did not have a significant impact on the probability of harm occurring.

There are several assumptions of the modelling upon which these results depend. For example, these results assume that the Grand and Thames river populations are stable, which cannot be confirmed without sampling data collected over multiple years following a structured monitoring design. If the Grand and (or) Thames river populations are declining, the magnitude of impacts occurring as a result of removals could be significantly worse. As well, an even sex ratio was assumed in simulations. If sample catches are biased toward males, more removals would be needed to reach the target number of females included in simulations, which may increase the amount of potential harm to the source populations.

Situations could occur where too many individuals are removed from a particularly important location for the population, such as important breeding individuals. Identifying critically important locations for Eastern Sand Darter is challenged by a lack of knowledge on metapopulation structure and breeding behaviour in the wild. Removing individuals from these areas could disproportionately affect population growth rates, particularly if individuals occupying these locations are more fit and contribute disproportionately to the population. The removal simulations assumed that age-1+ females were removed randomly throughout its distribution rather than removing a larger proportion of individuals from the most abundant sites to reduce the probability of significant harm at individual locations.

The probability of Eastern Sand Darter abundance in the Ausable River or Big Otter Creek growing larger than criteria related to  $MVP_{99\%}$  improved with the total number of individuals introduced and recipient location carrying capacity (Table 13; Figure 18). Probabilities of reaching the  $MVP_{99\%}$  thresholds were higher in the Ausable River than Big Otter Creek due to the greater overall habitat availability and, therefore, carrying capacity (Figure 14). Carrying capacity in the Ausable River and Big Otter Creek were based on estimated densities of Eastern Sand Darter from the Grand and Thames rivers and a resampling procedure of the proportion of

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total habitat suitable for the species. These are the most uncertain results from the modelling given the additional assumptions related to intraspecific life-history differences, namely the assumption that reintroduced population dynamics will mirror those of the source population.

Criteria for evaluating success and harm to populations resulting from reintroduction efforts were based on  $MVP_{99\%}$  estimates. The general recommendation for avoiding demographic harm to wild source populations is to ensure that there is a surplus of individuals. However, the challenges of estimating true abundance of rare, freshwater fish species causes significant uncertainty around whether a surplus exists and how large it may be. As a result of this uncertainty, empirically based thresholds of abundance to allow removals from a source population cannot be made and, instead, the results of population models that incorporate uncertainty must be used to inform decisions.

## **2.2. INFORMATION IN SUPPORT OF ESTIMATING THE PROBABILITY OF ESTABLISHING A SELF-SUSTAINING POPULATION OF EASTERN SAND DARTER IN THE RECIPIENT LOCATIONS**

There are many factors that can influence the probability of establishing a population for a species in an area where it was previously extirpated (i.e., ecological feasibility). Considering the role of these factors in advance of the reintroduction effort can help maximize the probability of reintroduction success. Broadly, these factors can be grouped into population, habitat (abiotic and biotic), and threat related factors. Below, 22 questions are presented to better understand how these factors may influence the probability of successful reintroduction for Eastern Sand Darter in southwestern Ontario (Table 14). Following these questions, the best available information for answering these questions is presented. These questions were developed and structured for use in a modified-Delphi process, where experts provide answers to the questions along with a measure of uncertainty based on their interpretation and evaluation of the question. This process was facilitated during the CSAS meeting, with the results presented in the Science Advisory Report (DFO 2025).

Table 14. 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.  $MVP_{99\%}$  = Minimum viable population size (99%).

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 $MVP_{99\%}$ 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 $MVP_{99\%}$ 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 $MVP_{99\%}$ 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 $MVP_{99\%}$ 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 $MVP_{99\%}$ in Big Otter Creek?
5a	Population	Thames River	What is the probability that population abundance is greater than 25,000 age-1+ individuals?

No.	Category	Focal location	Question
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?
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?

No.	Category	Focal location	Question
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 $MVP_{99\%}$ ?
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 $MVP_{99\%}$ ?
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 $MVP_{99\%}$ ?
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?
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?

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<b>No.</b>	<b>Category</b>	<b>Focal location</b>	<b>Question</b>
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?

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## Source population considerations

The recovery strategy for the Eastern Sand Darter Ontario DU identified several considerations prior to initiating reintroduction that were related to source and recipient populations (DFO 2012). This included the need to ensure that “*removal of individuals from source populations should not negatively affect the status of these populations*” and that “*source populations possess a high level of genetic diversity and genetic composition developed under similar historic conditions as the repatriation site*” (DFO 2012). Guidance from the American Fisheries Society similarly identifies the need to select a ‘*source population that is genetically fit*’ (Austen 2022). Ensuring that these characteristics are fulfilled requires estimates of population abundance, size structure, and trajectory, along with studies of genetic structure within and across populations. Below, background information is presented to inform answers to questions presented in Table 14.

### **Questions 1 and 2: Grand River population abundance and the probability of harm resulting from removals**

Indices of population abundance for age-1+ Eastern Sand Darter were calculated by extrapolating model-estimated densities across areas of potentially suitable habitat in the Grand River. Significant uncertainty in abundance estimates was caused by a limited understanding of the true amount of suitable habitat in both potential source rivers, including the amount of suitable habitat and density estimates across wadable and non-wadable habitats. Assuming the minimum and maximum proportion of total area with suitable habitat, and across wadable and non-wadable habitats, median estimated population abundance was 41,965 (mean = 48,011) or 119,159 (mean = 135,891) age-1+ individuals, respectively (Table 6).

Significant harm to Eastern Sand Darter populations as a result of removing individuals for reintroduction is contrary to the fundamental objective of improving survival and recovery for the species. Accordingly, the status of source populations should not be negatively affected by removals (DFO 2012). Defining when significant harm has occurred is challenging. Here, significant harm to the source population was defined as a decrease in population abundance below mean  $MVP_{99\%}$  (16,651 age-1+ females), and the lower confidence intervals of  $MVP_{99\%}$ , during or 10 years after removals have occurred. This implies that the probability of source population extirpation should never exceed 1% over the next 100 years throughout and following the removal efforts.

Assessing whether the Grand River population could withstand removals without reducing the population below  $MVP_{99\%}$  would benefit from long-term monitoring data to evaluate population trajectory, along with prior studies or pilot experiments evaluating the effects of removals on Eastern Sand Darter populations or populations of closely related species. Such studies could help reduce uncertainty around, for example, population growth rates and rates of translocation mortality. However, both types of studies are unavailable. As a result, determining whether the Grand River population can withstand maximum annual removals is reliant on model-based projections that incorporate several forms of uncertainty.

Age-1+ population abundance in the Grand River often fell below mean  $MVP_{99\%}$  during the removal simulations, but the magnitude of removals had a minimal effect on the probability of falling below mean  $MVP_{99\%}$  (Table 12). These model results were bounded by high uncertainty, from the uncertainty in initial model-generated density estimates (Figure 7) and extrapolation of abundance based on an uncertain availability of suitable habitat (Figure 9; Figure 12), to the population models that incorporated various forms of uncertainty when simulating removals and introductions.

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### Questions 3 and 7: Influence of population-specific life-history characteristics on establishing a self-sustaining population

Significant variation was incorporated into the population modelling around life-history parameters for evaluating the effects of removals due to uncertainty in the true values (Table 10). Consequently, the results of removals and additions 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.

An equal sex ratio has been identified as ideal for performing reintroduction of freshwater fishes, as an increased number of individuals are likely needed to compensate for imbalances (Williams et al. 1988). Imbalances in sex ratio can occur for several reasons such as inbreeding, differential mortality between sexes, or due to environmental pollutants (Fryxell et al. 2015), as well as sampling biases. Quantifying sex ratio is challenging from a sampling perspective, requiring consideration of the time, location, and frequency of sampling, and the number of individuals captured across size classes. Moreover, lethal sampling of individuals is required for assigning sexes to Eastern Sand Darter. In the population models used to estimate  $MVP_{99\%}$ , sex ratio was described at the egg stage (Equation 16) and assumed to be 50:50. A bias towards males in the sex ratio would increase estimates of  $MVP_{99\%}$  and therefore increase the relative harm experienced by the source population from removals.

Quantifying adult population sex ratio has not been performed for Eastern Sand Darter in the Grand River, with differing values reported for other populations (1:1 female to male; Spreitzer 1979; 2.54:1, Finch 2009). Sex ratio was quantified for 145 individuals from the Thames River obtained as a result of incidental mortalities or retrieved from predator gut contents in June and July of 2007 (Finch 2009). Based on these individuals, there was a 2.54:1 female to male ratio observed (Finch 2009, Finch et al. 2013). The mechanism underpinning the biased adult sex ratio observed in 2007 is unknown and may have been caused by opportunistic sampling. If this ratio reflects the true ratio in nature, the probability of successful reintroduction given a particular number of additions would be greater than what was presented in the reintroduction modelling scenarios, assuming that the number of males does not impact reproductive fitness of the population. Nevertheless, it seems likely that this ratio is a result of sampling bias.

Body size has been identified as an important consideration for improving success of fish introductions (e.g., Gunn et al. 1987, Diana and Wahl 2009). Larger individuals have a greater ability to avoid predation, locate prey, and are generally more fecund than smaller conspecifics (Morita and Takashima 1998, Barneche et al. 2018). As such, populations with larger individuals may be more suitable for reintroduction efforts, though body size may be less relevant for small-bodied species like Eastern Sand Darter. Quantifying differences in size of individuals between populations requires length data and can benefit from age measurements to allow differentiation between life-stages. During most sampling by Fisheries and Oceans Canada for Eastern Sand Darter in Ontario, length measurements are recorded for every individual captured that correspond to dates of sampling and other habitat characteristics. Length-at-age relationships quantified for the Thames River population suggest that age-1+ individuals are susceptible to capture by traditional gear types based on their size, and that there are minimal differences in total length between adult age-1+ year-classes (Figure 19).

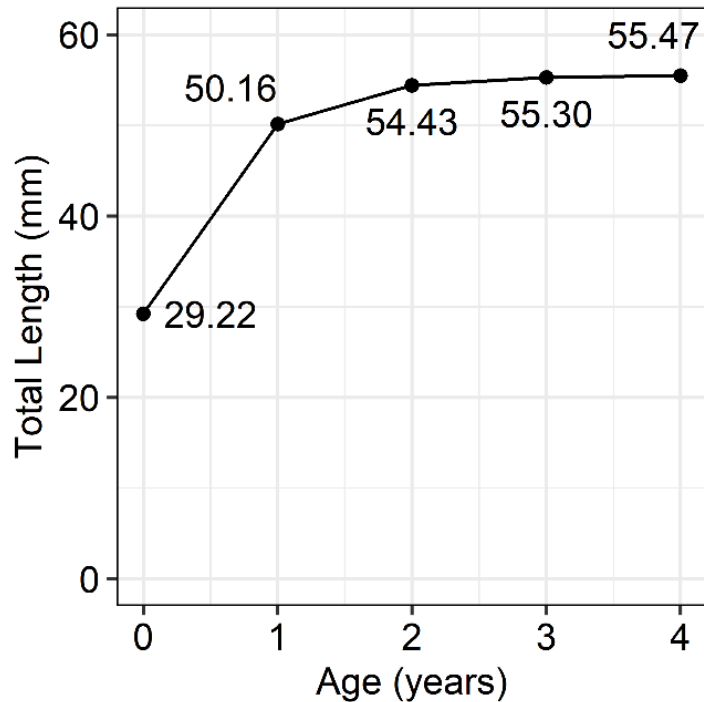


Figure 19. von Bertalanffy growth curve for Eastern Sand Darter generated using data from the lower Thames River collected in 2006 and 2007. Total length is calculated using the formula:  $TL(mm) = 55.52(1 - e^{-1.59(Age+0.47)})$  (Finch et al. 2013).

Total length (TL) data for Eastern Sand Darter collected by Fisheries and Oceans Canada and Dextrase (2013) were used to evaluate differences between individuals caught in the Grand and Thames rivers. Data used for this comparison span 17 (Thames River; 2006-2022) and 18 years (Grand River; 2007-2022) and included 997 individuals captured from the Thames River and 3,630 individuals captured from the Grand River. Only fishes greater than 29.22 mm TL (Figure 19) were included in this comparison to avoid problems with catchability of smaller fish. Differences in average TL of age-0+ Eastern Sand Darter were statistically significant between the Grand River (53.65 mm  $\pm$  8.18 SD) and Thames River (52.49 mm  $\pm$  7.82 SD;  $t_{1839.5} = 3.85$ ,  $p < 0.001$ ), although the biological significance of the difference is unknown. The definitive cause of this difference is also unknown, but a working hypothesis is that the reduced water clarity in the Thames River relative to the Grand River could cause differences in predation success as Eastern Sand Darter is a visual predator of benthic invertebrates.

Measurements of Eastern Sand Darter TL aggregated by month per river demonstrates the recruitment of juveniles to lengths susceptible to seine nets in June and July, leading to a single mode in September (Figure 20). The lack of age-0+ captures in June in the Thames River could be the result of several factors. For example, spawning could occur slightly later in the Thames River than the Grand River or juveniles in the Thames River could grow slower than those in the Grand River. Alternatively, the lack of captures could be a result of the sampling period in relation to thermal factors (e.g., growing degree days in sampling year). Many more individuals were captured in June in the Grand River than the Thames River (Figure 20), and juveniles < 30 mm were only captured in the Grand River in 2013, a year without length data from the Thames River. Ultimately, there is uncertainty about whether differences in growth between the Grand and Thames river populations would lead to a difference in establishment success at a recipient location.

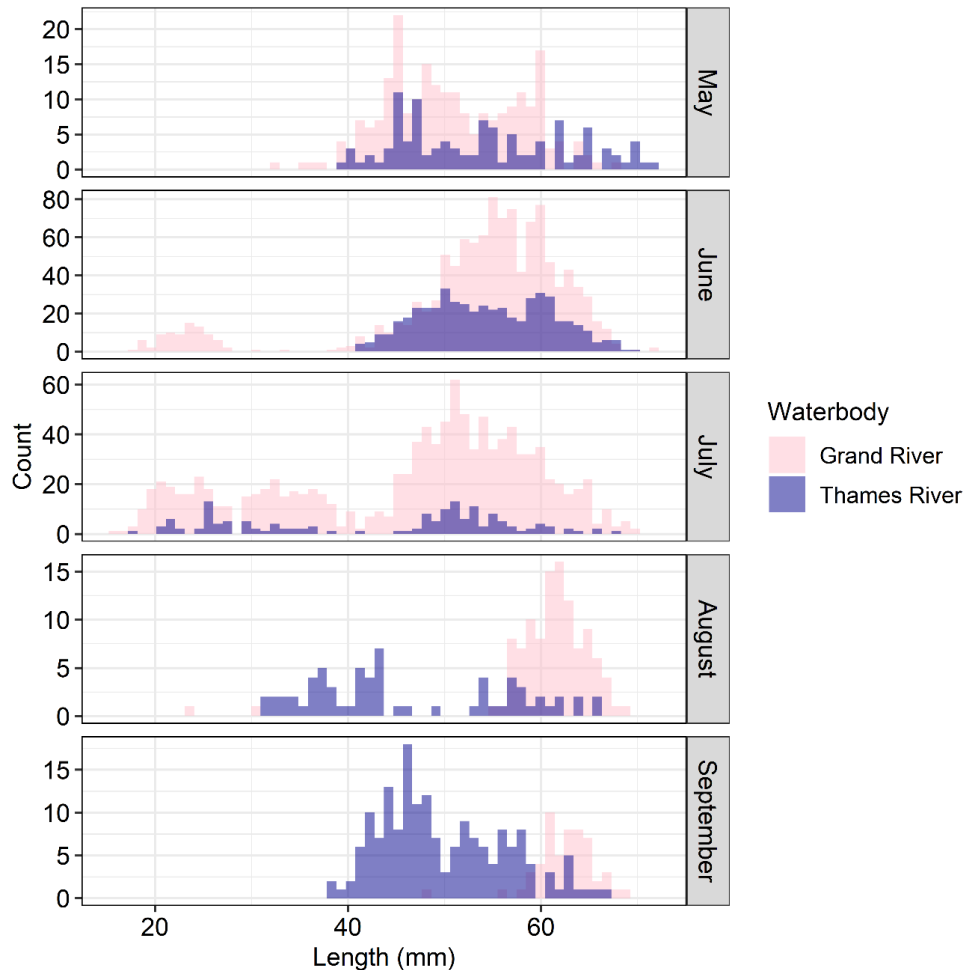


Figure 20. Length-frequency distribution of Eastern Sand Darter collected from the Grand ( $n = 3,630$  individuals; 2007-2022; pink) and Thames ( $n = 997$  individuals; 2006-2022; purple) rivers by DFO (2013) and Dextrase (2013). Note the differences in scales of the y-axis between panels.

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

A source-population selection framework was previously developed (Meffe 1995, Reisenbichler et al. 2003, Houde et al. 2015) and presented in Lamothe et al. (2023) that outlines three related approaches to select the appropriate source population when multiple populations are available: ancestry matching, environmental matching, and the use of adaptive potential (Figure 21). Ancestry matching describes the selection of source populations that share genetic similarity to the extant or historically present population, which assumes that shared genetic variation will confer adaptation to environmental conditions at the recipient site of reintroduction. This is generally the most preferred approach, but cannot be implemented for Eastern Sand Darter in the Ausable River or Big Otter Creek due to a lack of historical knowledge of genetic variation in those systems. Environmental matching describes the selection of a source population based on the similarity of habitat conditions between recipient and source locations and is generally considered to be the second-best approach to source population selection. Finally, the adaptive potential approach seeks to translocate individuals from multiple populations to provide the best

potential to adapt to conditions at the recipient site (i.e., experimental population; Kreuger et al. 1981; Figure 21).

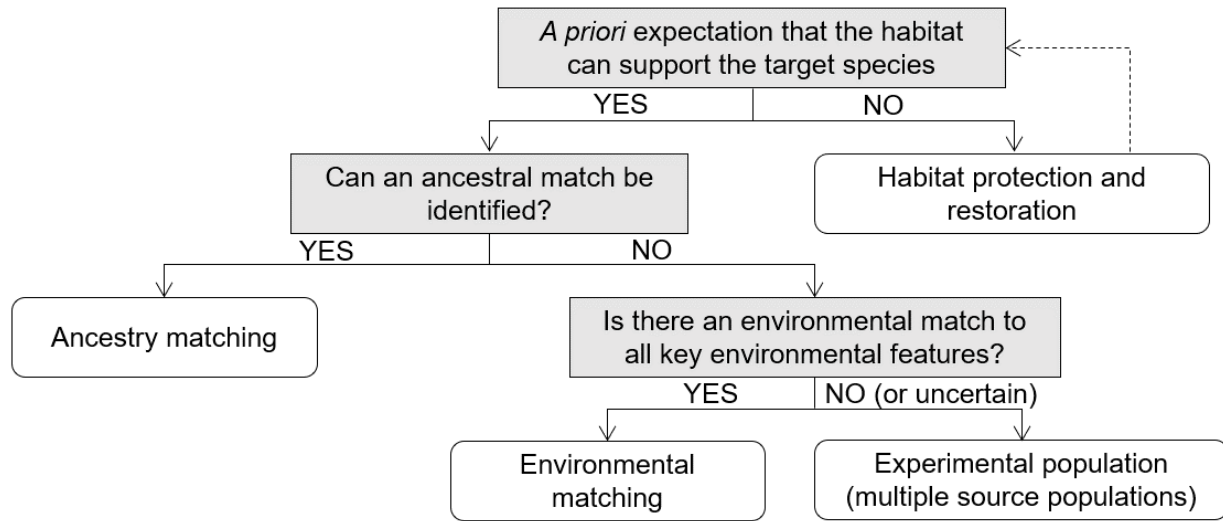


Figure 21. A source population selection framework for conservation translocation that requires knowledge of abiotic and biotic requirements for the species being considered. Originally presented in Lamothe et al. (2023).

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, Ginson et al. 2015). This suggests that populations in relatively close geographic proximity are likely most closely related because of shared post-glacial dispersal history. 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 (Ginson et al. 2015). Gene flow was observed among sites in the Grand and Thames rivers, resulting in a lack of significant isolation by distance (Ginson et al. 2015). These results imply significant within-river mixing despite including sites separated by dams in the analysis; however, given the recent construction of dams relative to the evolutionary history of Eastern Sand Darter in Ontario, observed differentiation between individuals above and below dams is unlikely. There have been no studies to determine if differentiation patterns across the genome are associated with different environments and might represent patterns of adaptive divergence.

#### **Questions 5 and 6: Thames River population abundance and the probability of harm resulting from removals**

Similar to the Grand River, monitoring of Eastern Sand Darter in the Thames River has been performed to fulfill different objectives and has not been replicated using standardized methods. Moreover, less effort has been dedicated to monitoring populations in the Thames River than the Grand River over the last decade. Model-generated density estimates for the Thames River population were based on data collected in 2006, with a limited understanding of the amount of suitable habitat available in the system, and no consideration of trajectory over time. Estimates of abundance for the Thames River population (Figure 12, Table 7) were below mean  $MVP_{99\%}$  in approximately 22% of the simulations prior to any removals.

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## Habitat considerations

Most studies that describe Eastern Sand Darter habitat use correlative associations between point habitat measurements and observed species occurrence or abundance (e.g., Drake et al. 2008, Dextrase et al. 2014, Reid and Dextrase 2017). Such habitat associations provide information on habitat use or habitat selection, but not necessarily habitat requirements (Rosenfeld 2003). Quantifying habitat requirements entails direct manipulation or experimentation to understand how survival, growth, reproduction, and (or) physiology are influenced by different habitat characteristics. Few studies have sought to quantify habitat requirements for Eastern Sand Darter (Firth et al. 2021a, Firth et al. 2023). Nevertheless, consistent observations of species within habitats having particular habitat conditions provides evidence of the importance of those habitat conditions for the ongoing occurrence of the species.

Habitat matching describes the process of comparing habitat characteristics from disparate locations. Habitat matching can provide information on whether habitat conditions in potential recipient locations are similar to habitats that support an Eastern Sand Darter population. When the data are available, different aspects of habitat characteristics important for the species can be compared between potential source and recipient locations including the mean, variance, and overlap in distributions of measurements (Figure 22).

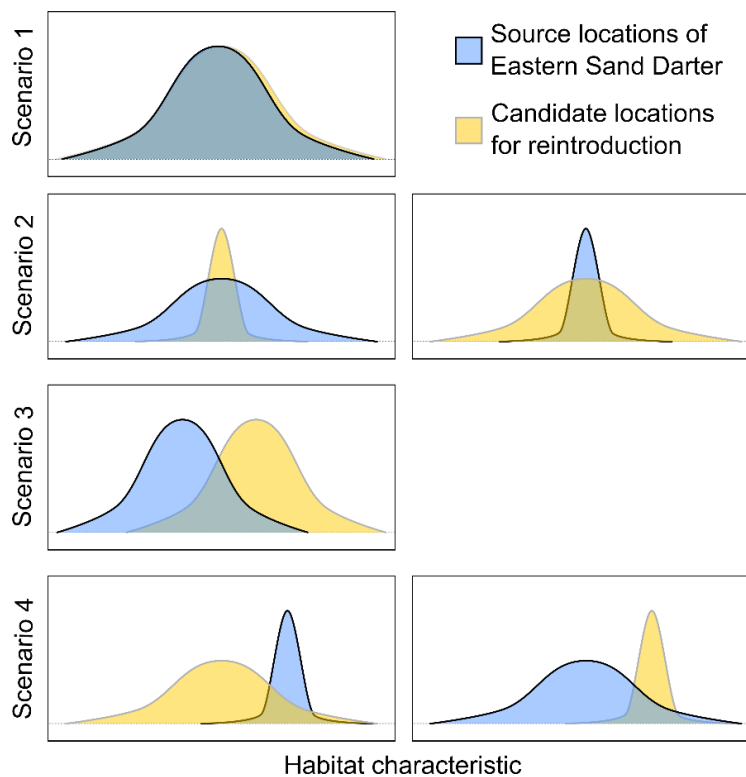


Figure 22. Conceptual relationship of habitat comparisons between source locations of Eastern Sand Darter (blue) and candidate locations for reintroduction (yellow). Scenario 1: Mean and variance match; Scenario 2: Mean match, variance mismatch; Scenario 3: Mean mismatch, variance match; Scenario 4: Mean and variance mismatch.

Four scenarios can arise when comparing habitat conditions between source and recipient locations (Figure 22). First, habitat conditions could be similar at sites with and without the species, with similar mean and variance observed between locations. Although unlikely, in this scenario it is possible that the habitat occupied in the source location could be contained at one

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end of the distribution relative to the habitat where reintroduction is proposed in the recipient location, and vice versa. In the second scenario, the mean values for a habitat variable may be similar between locations with Eastern Sand Darter detections and potential recipient locations, but the variance in locations with Eastern Sand Darter detections may be larger than potential recipient locations (Figure 22). This situation could imply that only a subset of propagules from the source location may be well matched for reintroduction. In the opposite manner, the potential recipient locations may show greater variation than sites where Eastern Sand Darter has been detected, suggesting that only a narrow range of habitat conditions in the recipient location may match well to the source. In the third scenario, mean values of a habitat variable could differ between locations, where the variance in measured habitat characteristic is similar or different (Figure 22). When means vary, this could imply that only a subset of propagules from the source location may be well matched for reintroduction; furthermore, in the fourth scenario, differences in mean and variance could suggest that an even smaller subset of propagules may be well-suited for reintroduction. Such approach to habitat matching does not consider individual plasticity to habitat conditions and therefore careful interpretation is recommended.

Below, a review of habitat requirements and associations for Eastern Sand Darter is provided, followed by an evaluation of habitat quantity requirements, and an analysis of habitat matching between potential recipient systems and habitats that support Eastern Sand Darter in the Grand and Thames rivers.

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

Eastern Sand Darter is a habitat specialist known to occupy areas with a high proportion of sand and fine gravel substrate. Research on southwestern Ontario populations 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, Dextrase et al. 2014; Figure 6, Figure 11). For example, research from the lower Thames River, Ontario indicated that the size of Eastern Sand Darter in its first year was positively related to the dominance of sand substrate, with individuals in sites dominated by silt being significantly smaller than individuals at sites dominated by sand (Figure 23; Drake et al. 2008). As well, a positive relationship between the occurrence and abundance of Eastern Sand Darter in the Grand and Thames rivers with the proportion of sand and fine gravel at a survey site has been previously demonstrated (Figure 23; Dextrase 2013, Dextrase et al. 2014), including in this document (Figure 6, Figure 11). Habitat selection trials in the laboratory using 20 Eastern Sand Darter captured from the lower Elk River, West Virginia demonstrated that out of 84 observations of fossorial behaviour, individuals primarily selected fine (0.125–0.25 mm), medium (0.25–0.50 mm), or coarse sand (0.5–1.0 mm), and avoided very coarse sand (1.0–2.0 mm; Thompson et al. 2017).

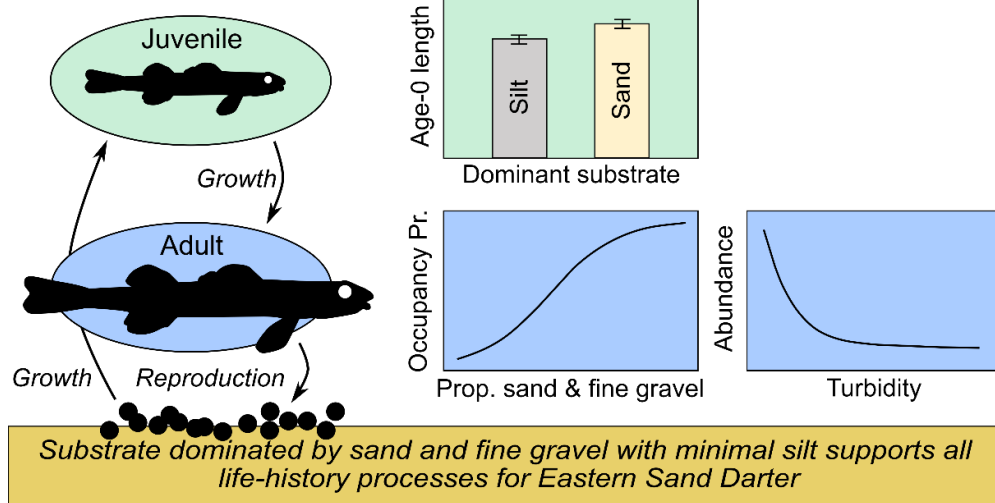


Figure 23. Representation of habitat niche for juvenile (< age-1) and adult (age-1+) Eastern Sand Darter. Blue and green indicate adult and juvenile life-stages, respectively.

The physiological requirements of Eastern Sand Darter have recently been assessed using streamside experiments in the Grand and Thames rivers (Firth et al. 2021a, 2023). Thermal tolerance was quantified using metrics of critical thermal maximum ( $CT_{max}$ ) and agitation temperature ( $T_{ag}$ ).  $CT_{max}$  is the temperature at which individuals lose locomotory control (Cox 1974) and likely represents death in the wild. Agitation temperature describes the temperature at which locomotory agitation is observed in response to temperature increase and is considered the temperature when individuals begin searching for thermal refugia (McDonnell and Chapman 2015, Firth et al. 2021a). Results from streamside experiments in the Grand and Thames rivers suggest a relatively high  $CT_{max}$  and  $T_{ag}$  for Eastern Sand Darter indicating high physiological variation. Moreover, a high acclimation response ratio was observed suggesting that the species may not be in immediate danger from warming temperatures (Firth et al. 2021a).

In addition to physiological effects of temperature, oxygen tolerance was assessed for Eastern Sand Darter in the Thames and Grand rivers (Firth et al. 2023). Results indicated that Eastern Sand Darter was tolerant of low oxygen (< 1 mg/L), similar to the highly hypoxia tolerant Swamp Darter (*Etheostoma fusiforme*; Ultsch et al. 1978). However, there was large individual variation in hypoxia tolerance across seasons, which may imply that some individuals have more opportunities to adapt to increases in temperature and decreases in oxygen. Moreover, experimental trials have also demonstrated no effect of turbidity on  $CT_{max}$ ,  $T_{ag}$ , metabolic rate, or hypoxia tolerance for Eastern Sand Darter in the Grand and Thames rivers (Firth et al. 2021a, Firth et al. 2023). Water clarity is among the most important variables for predicting Eastern Sand Darter abundance in the Grand and Thames rivers, where sites with high numbers of Eastern Sand Darter captures tended to have greater water clarity (Dextrase 2013; Figure 6, Figure 11, Figure 23).

Experimental work has not been performed to understand physiological tolerances to river velocity. Contradictory results have been found on the importance of river velocity for Eastern Sand Darter, with some studies demonstrating a negative relationship with species occurrence (Finch 2009) and others suggesting that river velocity is of lesser importance (Poos et al. 2008, Dextrase 2013). Given the correlation between river velocity and substrate composition, the availability of sand substrate is contingent on the appropriate river velocity; rivers with high

velocity will often contain substrates with larger particle sizes (e.g., cobble, boulders) whereas rivers with slow velocity can accumulate finer particles (e.g., silt).

There has been limited research directed toward understanding the habitat requirements for early life-history stages of Eastern Sand Darter. Previous research suggests that spawning occurs openly in the sand and fine gravel substrates during late spring to summer, when water temperature ranges between 14.4–25.5 °C (Williams 1975, Johnston 1989, Simon and Wallus 2006, DFO 2014). Age-0 Eastern Sand Darter have been detected co-occurring with age-1+ individuals, while also occurring at sites where no age-1+ individuals were detected.

### Eastern Sand Darter habitat-quantity requirements

The habitat area required to sustain an Eastern Sand Darter population is poorly understood. Modelling in Recovery Potential Assessments for SARA-listed freshwater fish species often includes estimates of minimum area for population viability (*MAPV*), which describes the amount of suitable habitat required for supporting a population at *MVP*<sub>99%</sub> (Vélez-Espino et al. 2010). Here, *MAPV* was calculated as the distribution of *MVP*<sub>99%</sub> estimates for Eastern Sand Darter divided by the distribution of median density estimates from the Brantford and Caledonia sections of the Grand River, and the Thames River (Figure 24). It was assumed that juveniles and adults occupied the same habitats, as both life-stages have been captured together.

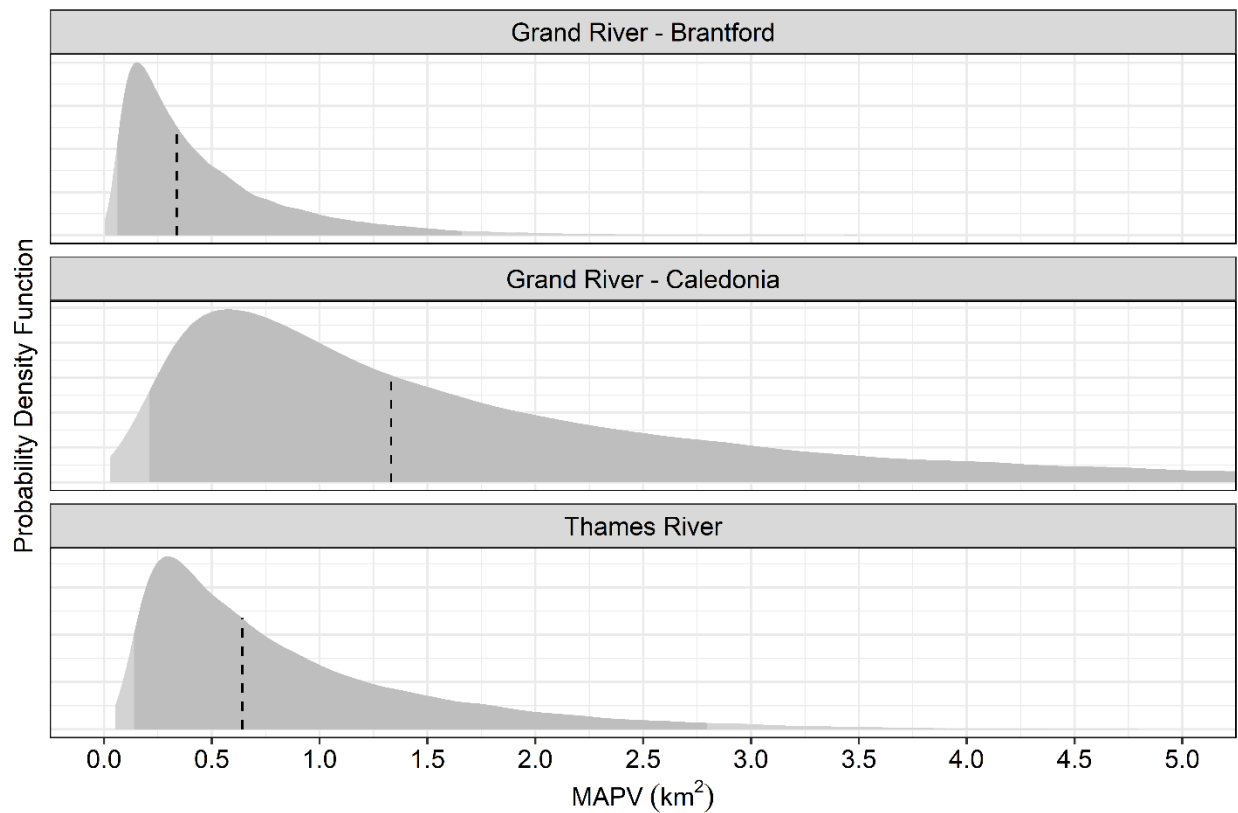


Figure 24. Minimum area for population viability (*MAPV*; km<sup>2</sup>) of Eastern Sand Darter in the Brantford and Caledonia sections of the Grand River, and the Thames River. Dashed lines indicate median values. Dark grey indicates 95% confidence interval.

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

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estimates from the Thames River (Figure 24). Recall that total habitat was estimated at 2,301,090 m<sup>2</sup> in the Ausable River. Approximately 58% of the total habitat would have to be identified as suitable, and be occupied, for Eastern Sand Darter to attain *MAVP* if the Caledonia Grand River densities occurred. Approximately, 28% or 15% of the total habitat would have to be identified as suitable, and be occupied, to attain *MAVP* if densities neared those in the Thames River or Brantford section of the Grand River, respectively.

Total habitat was estimated at 1,134,382 m<sup>2</sup> in Big Otter Creek. All of the total habitat would have to be identified as suitable, and be occupied, for Eastern Sand Darter to attain *MAVP* if the Caledonia Grand River density is anticipated. Approximately 56% of the total habitat would have to be identified as suitable and occupied to attain *MAVP* if density neared the Thames River population. Finally, 30% of the total habitat would have to be identified as suitable and occupied to attain *MAVP* if density neared the Brantford section of the Grand River.

### **Habitat-matching methods**

Habitat matching was performed for several habitat characteristics that have the potential to affect persistence of 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. Differences in the distribution of habitat measurements were tested using a Fligner-Killeen Test of Homogeneity of Variance (Fligner and Killeen 1976, Conover et al. 1981) and differences in mean values between rivers were tested using a Kruskal-Wallis Rank Sum Test (Kruskal and Wallis 1952). A Wilcoxon Rank Sum Test with a Bonferroni correction was used to make pairwise comparisons of mean values between rivers when a Kruskal-Wallis Test indicated significance (Conover and Iman 1981). Non-parametric approaches were chosen to avoid distributional assumptions. In addition, overlap in kernel densities of habitat characteristics was estimated between potential recipient locations and sites where Eastern Sand Darter was captured in the Grand and Thames rivers. A Gaussian kernel was used to generate the probability density functions, where the bandwidth was set to two times the standard deviation of the kernel to avoid over-fit distributions.

Multiple datasets were used for making comparisons. In the first, all possible observations were included where the particular variable of interest was measured (hereafter referred to as “All Data”). Data from the Ausable River were split into two geographically distinct sections, upstream of ‘The Cut’ to Highway 7 in Ailsa Craig and upstream of Ailsa Craig to the Exeter dam (Figure 13). The second data set was from Dextrase (2013), which remains the most extensive survey of Eastern Sand Darter. Due to the sample size from the Ausable River in Dextrase (2013;  $n = 24$ ; Table A1), analyses were performed for the entire system, where survey sites were located in both the middle and upper sections of the river. In addition to the two primary data sets, hourly time series data of water temperature were available for Big Otter Creek, the Grand River, and the Thames River from loggers deployed between 2019 and 2021. As well, targeted substrate and dissolved oxygen measurements were evaluated based on sampling in 2020 (Barnucz et al. 2022; Britney Firth, University of Waterloo, pers. comm.).

The results of habitat-matching analyses are presented below. Care is recommended when interpreting the results of these analyses given the different objectives, sampling periods, sample sizes, and approaches used across projects.

### **Question 9: Availability of suitable habitat in the Ausable River**

#### **Substrate**

Sand and fine gravel substrate is considered the most important habitat characteristic for supporting Eastern Sand Darter populations in the wild. There were significant differences in the

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mean and variance of percent sand measurements between the potential source populations and the Ausable River sections (Table 15; Table 16; Figure 25). Percent sand measurements in the middle and upper sections of the Ausable River were, on average, significantly lower than measurements taken in the Grand and Thames rivers where Eastern Sand Darter has previously been detected ( $p < 0.001$ ), with no difference observed between Ausable River sections ( $p = 1.000$ ). Based on the data from Dextrase (2013), there were no significant differences in the mean or variance of percent sand and fine gravel measurements in the Ausable River and sites in the Grand and Thames rivers where Eastern Sand Darter has previously been detected (Table 17; Table 18). There was significant heterogeneity in variance (Table 15) and significant differences in mean percent silt between the Ausable River sections and potential source locations ( $p < 0.001$ ; Table 16). Generally, there was greater variation in silt cover in the Thames River measurements than measurements from the Grand River or Ausable River sections (Table 16), with the Ausable River having much greater average silt cover. Based on the data from Dextrase (2013), the Grand River had significantly lower silt and clay cover than the Ausable River ( $p = 0.047$ ; Figure 26), with no difference observed between the Ausable River and Thames River ( $p = 1.000$ ).

Table 15. Results of Fligner-Killeen's (FK) tests for homogeneity of variance of habitat measurements from the middle and upper sections of the Ausable River, and sites where Eastern Sand Darter was captured in the Grand and Thames rivers. Included are tests for when all data are included. Note:  $n$  = number of samples and  $p$  = significance level.

Data subset	Habitat variable	Grand River $n$	Thames River $n$	Middle Ausable $n$	Upper Ausable $n$	FK-statistic	$p$
All	Water temperature (°C)	173	541	85	58	18.22	< 0.001
All	Conductivity ( $\mu$ S/cm)	162	376	74	38	34.91	< 0.001
All	Turbidity (NTU)	86	180	45	17	162.51	< 0.001
All	Dissolved oxygen (mg/L)	136	282	57	29	35.00	< 0.001
All	River velocity (m/s)	88	72	36	17	57.07	< 0.001
All	% Sand	114	518	69	47	20.30	< 0.001
All	% Silt	114	518	63	37	8.96	0.030

Table 16. Results of Kruskal-Wallis chi-square ( $KW \chi^2$ ) rank sum test of habitat measurements from the middle and upper sections of the Ausable River and sites where Eastern Sand Darter was captured in the Grand and Thames rivers. Mean  $\pm$  SD (standard deviation) presented. Included are tests for when all data are included. Sample sizes are presented in Table 15. Note:  $p$  = significance level.

Data subset	Habitat variable	Grand River	Thames River	Middle Ausable River	Upper Ausable River	$KW \chi^2$	$p$
All	Water temperature (°C)	22.75 $\pm$ 4.46	22.50 $\pm$ 4.73	21.91 $\pm$ 3.27	22.09 $\pm$ 2.43	18.49	< 0.001
All	Conductivity ( $\mu$ S/cm)	792.96 $\pm$ 104.34	582.38 $\pm$ 124.67	471.44 $\pm$ 60.68	522.41 $\pm$ 134.15	348.77	< 0.001
All	Turbidity (NTU)	14.12 $\pm$ 38.23	140.24 $\pm$ 103.81	29.82 $\pm$ 18.79	10.06 $\pm$ 1.98	223.62	< 0.001
All	Dissolved oxygen (mg/L)	8.71 $\pm$ 1.85	8.84 $\pm$ 1.70	7.21 $\pm$ 1.30	6.32 $\pm$ 1.27	97.58	< 0.001
All	River velocity (m/s)	0.21 $\pm$ 0.20	0.64 $\pm$ 0.29	0.07 $\pm$ 0.08	0.14 $\pm$ 0.10	110.94	< 0.001
All	% Sand	64.74 $\pm$ 29.37	51.93 $\pm$ 32.17	25.14 $\pm$ 22.69	28.30 $\pm$ 24.23	86.03	< 0.001
All	% Silt	5.57 $\pm$ 11.73	9.84 $\pm$ 18.75	12.62 $\pm$ 15.16	12.30 $\pm$ 10.18	35.39	< 0.001

Table 17. Results of Fligner-Killeen's (FK) tests for homogeneity of variance of habitat measurements from the Ausable River, Big Otter Creek, and sites where Eastern Sand Darter was captured in the Grand and Thames rivers. Included are tests for when only data from Dextrase (2013) are included. Sample sizes (*n*) are provided for each variable per waterbody. Note: *p* = significance level.

Data subset	Habitat variable	Grand River <i>n</i>	Thames River <i>n</i>	Ausable River <i>n</i>	Big Otter Creek <i>n</i>	FK-statistic	<i>p</i>
Dextrase	Water temperature (°C)	34	66	24	14	17.46	< 0.001
Dextrase	Conductivity (µS/cm)	34	56	24	14	14.02	0.003
Dextrase	Water clarity (cm)	34	66	24	14	6.31	0.097
Dextrase	Dissolved oxygen (mg/L)	34	66	24	14	27.09	< 0.001
Dextrase	River velocity (m/s)	34	60	24	14	1.76	0.623
Dextrase	Mean substrate size (mm)	34	66	24	14	8.76	0.033
Dextrase	% Silt and clay	34	66	24	14	27.57	< 0.001
Dextrase	% Sand and fine gravel	34	66	24	14	5.30	0.151

Table 18. Results of Kruskal-Wallis chi-square (KW  $\chi^2$ ) rank sum test of habitat measurements from the Ausable River, Big Otter Creek, and sites where Eastern Sand Darter was captured in the Grand and Thames rivers. Mean  $\pm$  SD (standard deviation) presented. Included are tests for when only data from Dextrase (2013) are included. Sample sizes are presented in Table 17. Note: *p* = significance level.

Data subset	Habitat variable	Grand River	Thames River	Ausable River	Big Otter Creek	KW $\chi^2$	<i>p</i>
Dextrase	Water temperature (°C)	23.17 $\pm$ 3.01	23.48 $\pm$ 2.83	24.35 $\pm$ 1.22	21.04 $\pm$ 1.51	16.80	< 0.001
Dextrase	Conductivity (µS/cm)	772.62 $\pm$ 37.21	632.32 $\pm$ 56.09	494.50 $\pm$ 52.10	565.43 $\pm$ 21.65	102.50	< 0.001
Dextrase	Water clarity (cm)	75.65 $\pm$ 26.97	32.11 $\pm$ 30.49	32.25 $\pm$ 24.13	39.50 $\pm$ 8.83	44.67	< 0.001
Dextrase	Dissolved oxygen (mg/L)	8.86 $\pm$ 2.13	8.41 $\pm$ 1.23	6.26 $\pm$ 0.92	9.18 $\pm$ 0.62	45.07	< 0.001
Dextrase	River velocity (m/s)	0.16 $\pm$ 0.11	0.16 $\pm$ 0.10	0.08 $\pm$ 0.12	0.19 $\pm$ 0.11	19.27	< 0.001
Dextrase	Mean substrate size (mm)	19.51 $\pm$ 45.41	6.93 $\pm$ 8.48	10.57 $\pm$ 18.30	6.83 $\pm$ 12.38	7.49	0.058
Dextrase	% Silt and clay	2.04 $\pm$ 4.79	12.27 $\pm$ 17.03	11.05 $\pm$ 15.92	10.00 $\pm$ 12.52	12.52	0.006
Dextrase	% Sand and fine gravel	53.49 $\pm$ 33.72	66.62 $\pm$ 24.45	68.76 $\pm$ 26.97	74.52 $\pm$ 28.39	6.40	0.094

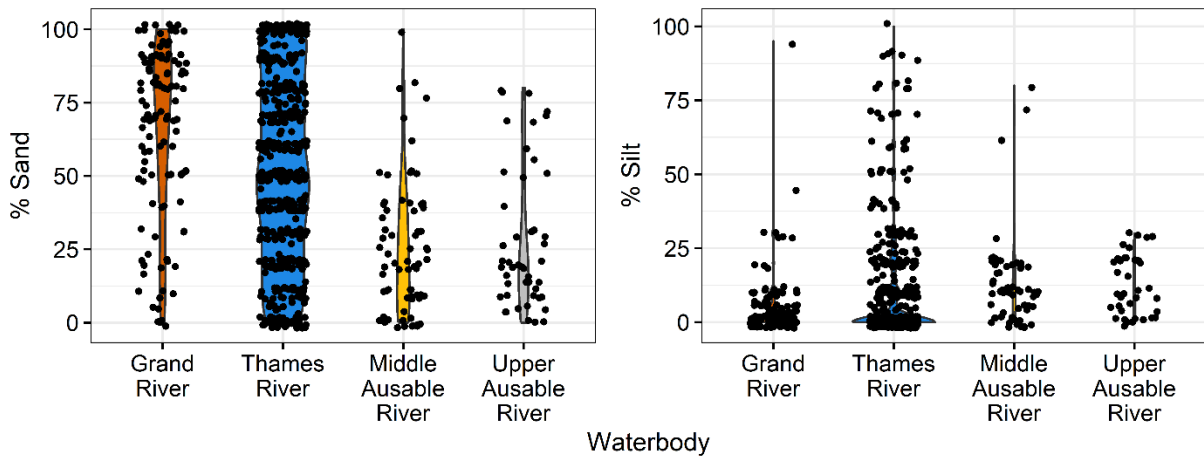


Figure 25. Percent sand and silt measurements at sites in the middle and upper sections of the Ausable River, and in the Grand and Thames rivers where Eastern Sand Darter was captured. Points indicate empirical measurements and are jittered for visual purposes. Violins (colours) were scaled proportionally to the number of observations per location. Sample sizes per variable per location are presented in Table 15.

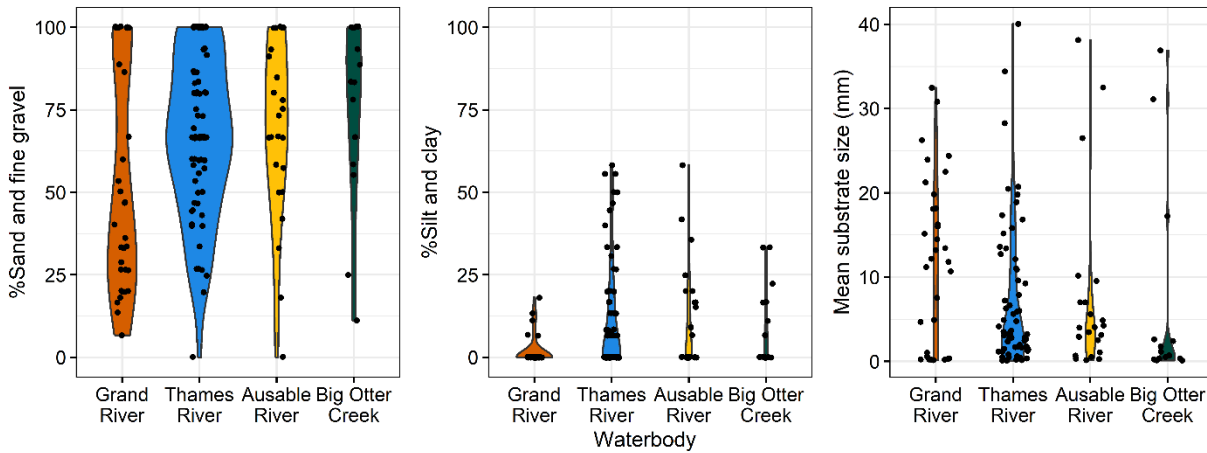


Figure 26. Percent sand and fine gravel, percent silt and clay, and mean substrate size (mm) at sites in the Ausable River and Big Otter Creek, and in the Grand and Thames rivers where Eastern Sand Darter was captured. Points indicate measurements from Dextrase (2013) and are jittered for visual purposes. Violins (colours) were scaled proportionally to the number of observations per location. Two observations were removed from the mean substrate size plot that were > 80 mm, one from the Ausable River and one from the Grand River. Sample sizes per variable per location are presented in Table 17.

### Water clarity

Water clarity is frequently described as having a positive influence on the occurrence and abundance of Eastern Sand Darter. The mean and variance in turbidity measurements were significantly different across rivers/sections (Table 15; Table 16). The Grand and Thames rivers had a greater range of turbidity measurements than the upper or middle Ausable River sections (Figure 27); however, sample sizes were relatively small for the Ausable River. Pairwise tests suggested significantly different ( $p < 0.001$ ) mean turbidity measurements between the middle Ausable River section and the Grand River, the middle Ausable River section and the Thames

River, the upper Ausable River section and the Thames River, and the middle and upper Ausable River sections. Mean turbidity was lowest in the upper Ausable River, followed by the Grand River and middle Ausable River, with the Thames River having the highest mean turbidity (Table 16). The middle and upper Ausable River sections had greater overlap with the Grand River than the Thames River in kernel density estimates of turbidity, with the middle Ausable River section showing greater overlap with the Thames River than the upper Ausable River section (Table 19). Based on the data from Dextrase (2013), the Ausable River had significantly lower water clarity than the Grand River ( $p < 0.001$ ), with no significant difference observed with the Thames River ( $p = 0.407$ ; Figure 28).

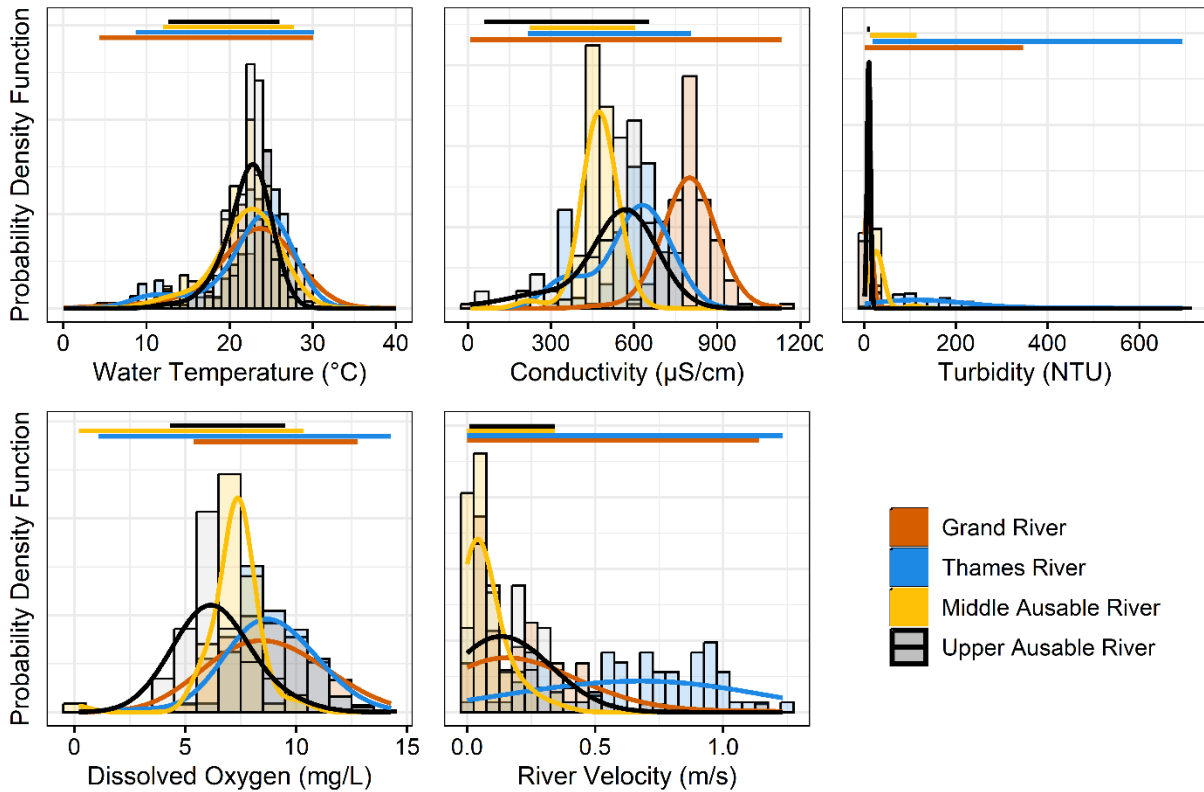


Figure 27. Histogram and kernel density plots for habitat measurements in the middle (yellow) and upper (grey and black) sections of the Ausable River, and at sites in the Grand (orange) and Thames (blue) rivers where Eastern Sand Darter was captured. Horizontal segments on the top of each plot indicate range of measured values per waterbody. Sample sizes per variable per location are presented in Table 15.

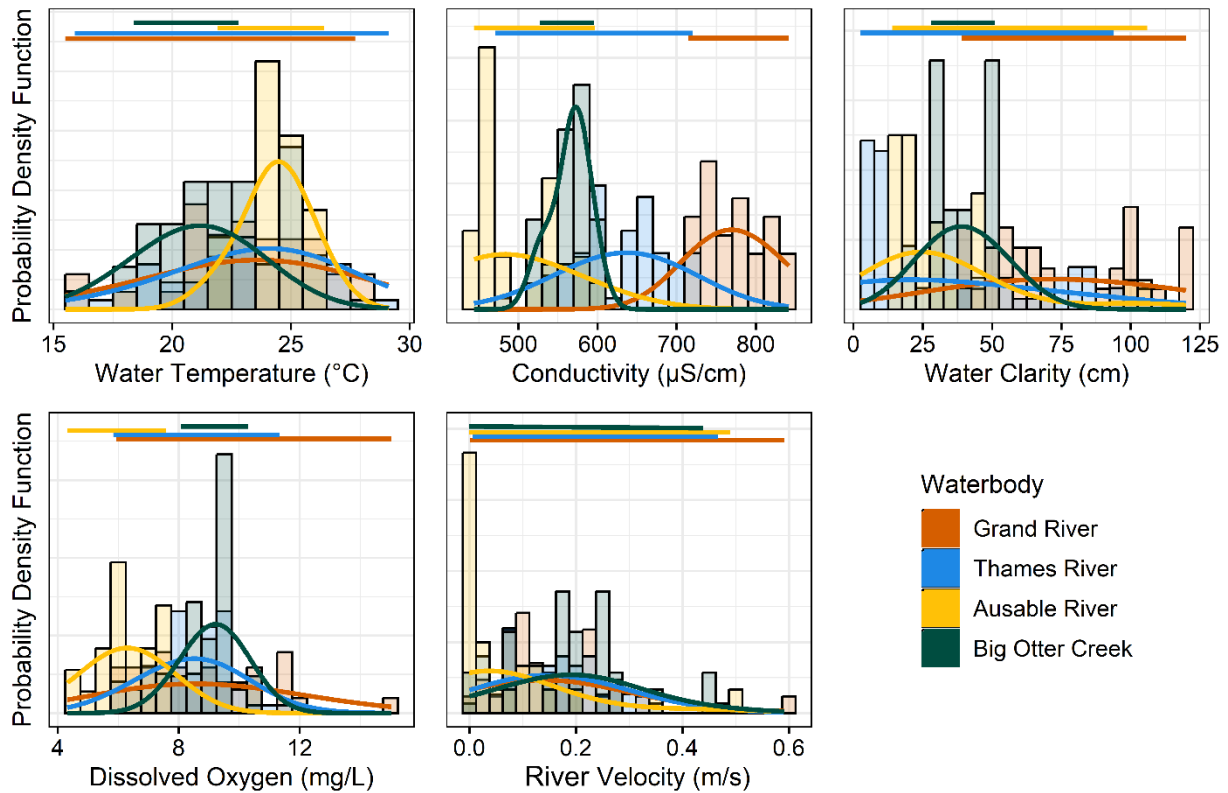


Figure 28. Histogram and kernel density plots for habitat measurements in the Ausable River (yellow) and Big Otter Creek (green), and in the Grand (orange) and Thames (blue) rivers where Eastern Sand Darter was captured. Horizontal segments on the top of each plot indicate range of measured values per waterbody. Plots only include data from Dextrase (2013). Sample sizes per variable per location are presented in Table 17.

Table 19. Percent overlap in kernel density estimates for habitat variables in the middle and upper sections of the Ausable River with measurements taken in the Grand and Thames rivers where Eastern Sand Darter was captured.

Data	Recipient Location	Habitat variable	Grand River	Thames River
All	Middle Ausable	Water temperature (°C)	84.27%	79.32%
All	Middle Ausable	Conductivity (µS/cm)	3.65%	32.65%
All	Middle Ausable	Turbidity (NTU)	36.18%	20.04%
All	Middle Ausable	Dissolved oxygen (mg/L)	52.60%	48.00%
All	Middle Ausable	River velocity (m/s)	50.48%	19.15%
All	Upper Ausable	Water temperature (°C)	72.99%	67.58%
All	Upper Ausable	Conductivity (µS/cm)	17.91%	71.35%
All	Upper Ausable	Turbidity (NTU)	59.26%	3.95%
All	Upper Ausable	Dissolved oxygen (mg/L)	51.88%	41.61%
All	Upper Ausable	River velocity (m/s)	80.66%	30.39%

Data	Recipient Location	Habitat variable	Grand River	Thames River
Dextrase	Ausable River	Water temperature (°C)	61.34%	66.44%
Dextrase	Ausable River	Conductivity (µS/cm)	2.03%	34.60%
Dextrase	Ausable River	Water clarity (cm)	45.24%	80.85%
Dextrase	Ausable River	Dissolved oxygen (mg/L)	46.89%	44.13%
Dextrase	Ausable River	River velocity (m/s)	65.20%	61.09%

### Other habitat characteristics

Other habitat characteristics like stream velocity and several water quality characteristics (temperature, conductivity, and dissolved oxygen) may influence the probability of successful reintroduction. Habitat matching results are provided below for each variable independently.

Based on either subset of data, the Ausable River had the lowest average river velocity (Table 16; Table 18). Significant pairwise differences in river velocity were observed between the middle Ausable River section and the Grand River ( $p < 0.001$ ), the middle Ausable River section and the Thames River ( $p < 0.001$ ), upper Ausable River and Thames River ( $p < 0.001$ ), and the middle and upper Ausable River ( $p = 0.042$ ). The middle Ausable River had the lowest average river velocity, followed by the upper section, the Grand River, and the Thames River (Table 16). The upper Ausable River showed greater overlap than the middle section with the potential source populations in kernel density estimates of river velocity (Table 19). When considering data from Dextrase (2013), overlap in kernel density estimates of river velocity were equivalent between the Grand (65.20%) and Thames rivers (61.09%; Table 19).

Significant heterogeneity in variance and differences in mean water temperature were observed between rivers/sections (Table 15; Table 16; Table 17; Table 18), with significant pairwise differences in mean temperature between the middle Ausable River section and the Thames River ( $p = 0.007$ ) and the upper Ausable River section and the Thames River ( $p = 0.007$ ). The middle and upper sections had slightly lower mean water temperature than the Grand and Thames rivers (Table 16). This is in contrast with the results from Dextrase (2013), which had a higher mean water temperature in the Ausable River than the Grand or Thames rivers (Table 18; Figure 28). Nevertheless, the range of values measured in the Ausable River fell within the measured range of values in the Grand and Thames Rivers for both data sets (Figure 27; Figure 28). There was approximately 72.99% and 67.58% overlap between kernel density estimates of water temperature in the upper Ausable River and sites where Eastern Sand Darter was previously detected in the Grand River and Thames River, respectively (Table 19). Greater similarity in conditions was observed between the middle Section and potential source populations; there was approximately 84.27% and 79.32% overlap between kernel density estimates of water temperature at sites where Eastern Sand Darter was previously detected in the Grand River and Thames River, respectively (Table 19).

Significant differences in the mean and variation of conductivity measurements were observed between rivers/sections (Table 15; Table 16; Table 17; Table 18). The upper and middle Ausable River sections had significantly lower conductivity ( $p < 0.003$ ), on average, than the Grand and Thames rivers (Table 16; Figure 27). With the wider range of measured values (Figure 27), there was greater overlap in kernel density estimates of conductivity measurements between the upper Ausable River section and potential source locations than with the middle Ausable River section (Table 19). Conductivity measurements were low in the Ausable River when considering data from Dextrase (2013), with minimal overlap with measurements from the Grand or Thames rivers (Table 19; Figure 28).

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Measurements of dissolved oxygen from the upper and middle Ausable River sections were significantly lower than in the Grand and Thames Rivers ( $p < 0.001$ ), with the middle Ausable River section having significantly higher mean dissolved oxygen than the upper section ( $p < 0.001$ ). Variance was heterogenous across rivers/sections (Table 15; Table 18), with the Thames River having the greatest range of measurements followed by the Grand River and the two Ausable River sections (Figure 27). Kernel density estimates of dissolved oxygen for the middle and upper sections overlapped with the Grand and Thames rivers by 41.61–52.60% (Table 19). The Ausable River had significantly ( $p < 0.001$ ) lower mean dissolved oxygen measurements than the Grand or Thames rivers when considering the Dextrase (2013) data (Table 18), where a 46.89% and 44.13% overlap in kernel density estimates of dissolved oxygen was observed between the Ausable River and the Grand and Thames rivers, respectively (Table 19).

#### **Question 10: Connectivity in the Ausable River for supporting all life-stages**

Identifying the degree of connectivity necessary to support a population requires an understanding of the life-history of the species and direction and magnitude of movement among and between self-sustaining populations. Minns (1995) developed an allometric relationship between home range size ( $HR$ ;  $m^2$ ) and body length (mm) for riverine species, where:

$$HR = 0.0545 \times L^{1.65}. \quad \text{Equation (23)}$$

Assuming a maximum total length of approximately 56 mm, home-range size for riverine populations of Eastern Sand Darter is  $42 m^2$ . Tracking studies on Eastern Sand Darter have not been performed to confirm this estimate. However, tagging was performed for Eastern Sand Darter in the Grand River (Cockshutt Bridge) to identify individuals that were used for physiological experiments (Gáspárdy and Drake 2021). During these trials, 282 individuals were tagged with 37 recaptures recorded (Gáspárdy and Drake 2021), suggesting minimal movement and a potentially small home range of the recaptured fish. Mark-recapture studies have been performed on species of similar life-history strategies. For example, a mark-recapture study on Rainbow Darter (*Etheostoma caeruleum*) in the Grand River, Ontario that occurred over one year (initial tagging in July 2014, recapture sampling in August 2014, November 2014, May 2015, and August 2015) indicated high site fidelity for the species; 85% of recaptures were within the riffle in which individuals were originally tagged with a maximum upstream recapture distance of 420 m. Among the fish recaptured outside of the initial tagging site in November 2014, most had moved upstream whereas the recaptures of individuals outside of the individual tagging site in May 2015 were mostly found downstream (Hicks and Servos 2017). Given the similar life-history of Eastern Sand Darter, it is likely that upstream and downstream movement of individuals occurs at a low rate, but the importance of this movement for the persistence of the population is unknown.

Studies on the genetic structure of Eastern Sand Darter have been more focused on larger-scale population differentiation than movements and dispersal within populations (Ginson et al. 2015, Walter et al. 2022). Nevertheless, the results of this research identified low within-river genetic variation among southwestern Ontario Eastern Sand Darter populations, with significant mixing occurring between individuals occupying different areas of the river (Ginson et al. 2015). The ephemeral nature of sand and fine gravel habitat may contribute to the mixing observed (Ginson et al. 2015) or may be the result of active dispersal or being forced downstream as a result of elevated flows.

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## **Question 11: Availability of suitable habitat in Big Otter Creek**

### **Substrate**

Variance in the percent cover of sand substrate was not significantly different across waterbodies (Table 20). Although a significant difference in mean percent cover of sand substrate was observed between waterbodies (Table 21), no significant pairwise difference was observed between Big Otter Creek and sites in the Grand River or Thames River with records of Eastern Sand Darter detections (Figure 29). The distribution of percent sand cover measurements was relatively uniform between 0 and 100% at sites in the Thames River with Eastern Sand Darter detections, while sites in the Grand River with Eastern Sand Darter detections trended toward having a higher percent sand coverage (Figure 29). A bimodal distribution was observed for percent sand measurements in Big Otter Creek (Figure 29). No significant differences were observed in the percent sand and fine gravel cover between Big Otter Creek and sites where Eastern Sand Darter were previously detected in the Grand and Thames rivers when considering data from Dextrase (2013; Table 18; Figure 26). Nevertheless, Big Otter Creek had the highest average percent cover of sand and fine gravel habitat ( $74.52 \pm 28.39$  SD) compared to any of the other focal systems (Table 18).

Table 20. Results of Fligner-Killeen's (FK) tests for homogeneity of variance of habitat measurements from Big Otter Creek and sites where Eastern Sand Darter was captured in the Grand and Thames rivers. Included are tests for when all data are included. Note:  $n$  = number of samples and  $p$  = significance level.

Data subset	Habitat variable	Grand River $n$	Thames River $n$	Big Otter Creek $n$	FK-statistic	$p$
All	Water temperature (°C)	173	541	55	0.33	0.849
All	Conductivity ( $\mu\text{S}/\text{cm}$ )	162	376	55	25.97	< 0.001
All	Turbidity (NTU)	86	180	55	154.25	< 0.001
All	Dissolved oxygen (mg/L)	136	282	55	17.12	< 0.001
All	River velocity (m/s)	88	72	46	38.61	< 0.001
All	% Sand	114	518	43	1.89	0.388
All	% Silt	114	518	43	4.03	0.134

Table 21. Results of Kruskal-Wallis chi-square ( $KW \chi^2$ ) rank sum test of habitat measurements from Big Otter Creek and sites where Eastern Sand Darter was captured in the Grand and Thames rivers. Mean  $\pm$  SD (standard deviation) presented. Included are tests for when all data are included. Sample sizes are presented in Table 20. Note:  $p$  = significance level.

Data subset	Habitat variable	Grand River	Thames River	Big Otter Creek	$KW \chi^2$	$p$
All	Water temperature (°C)	22.75 $\pm$ 4.46	22.50 $\pm$ 4.73	19.13 $\pm$ 4.19	41.34	< 0.001
All	Conductivity ( $\mu\text{S}/\text{cm}$ )	792.96 $\pm$ 104.34	582.38 $\pm$ 124.67	552.61 $\pm$ 58.56	293.78	< 0.001
All	Turbidity (NTU)	14.12 $\pm$ 38.23	140.24 $\pm$ 103.81	17.33 $\pm$ 13.04	217.04	< 0.001
All	Dissolved oxygen (mg/L)	8.71 $\pm$ 1.85	8.84 $\pm$ 1.70	9.50 $\pm$ 1.15	12.38	0.002
All	River velocity (m/s)	0.21 $\pm$ 0.20	0.64 $\pm$ 0.29	0.22 $\pm$ 0.11	84.80	< 0.001
All	% Sand	64.74 $\pm$ 29.37	51.93 $\pm$ 32.17	60.35 $\pm$ 28.23	16.26	< 0.001
All	% Silt	5.57 $\pm$ 11.73	9.84 $\pm$ 18.75	14.53 $\pm$ 10.79	21.71	< 0.001

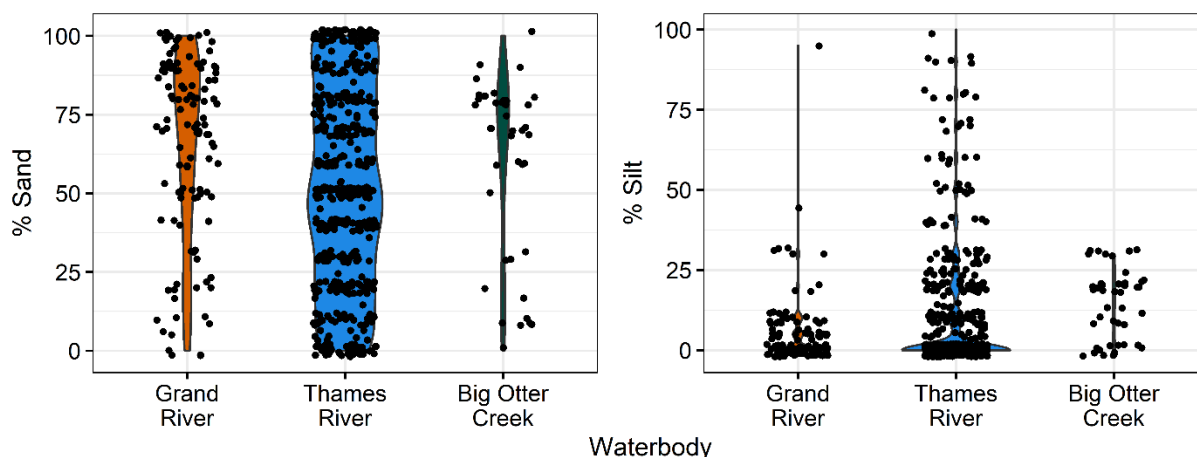


Figure 29. Percent sand and silt measurements at sites in Big Otter Creek, and in the Grand and Thames rivers where Eastern Sand Darter was captured. Points indicate empirical measurements and are jittered for visual purposes. Violins (colours) were scaled proportionally to the number of observations per waterbody. Sample sizes per variable per waterbody are presented in Table 20.

The average percent silt cover at a site was significantly different across waterbodies (Table 21; Figure 29). Average percent silt cover at sites in Big Otter Creek was significantly higher than sites in the Grand River ( $p < 0.001$ ) and Thames River ( $p < 0.001$ ) where Eastern Sand Darter detections have occurred, with no pairwise difference observed between sites sampled in the Grand and Thames rivers ( $p = 1.000$ ). Similarly, significant differences in average proportion of silt and clay coverage were observed across waterbodies based on the Dextrase (2013) data (Table 18). Here, however, differences were observed between the Grand and Thames River sites, where the Thames River sites had a higher average percent of silt and clay cover than Grand River sites (Table 18). Significant differences in mean substrate size variance were observed among waterbodies (Table 17), where the Grand River showed larger variation in mean substrate size than the Thames River or Big Otter Creek. The average mean substrate size was marginally significant across systems; mean substrate size was similar between Big Otter Creek and the Thames River, which was smaller than the average mean substrate size in the Grand River (Table 18).

### Targeted substrate sampling

Sampling in Big Otter Creek was conducted in 2020 to evaluate habitat conditions at six sites within and six sites beyond historical records of Eastern Sand Darter detections (Barnucz et al. 2022). Sampled sites primarily consisted of point bar areas within wadable pools. The dominant substrate class at the six sites within historical detection boundaries was medium sand ( $\geq 0.25$  mm –  $< 0.5$  mm), ranging from a mean of 26.0% to 61.2% composition at each site (Figure 30). Medium gravel or larger ( $\geq 8$  mm) was the second-most common substrate class at the two upstream-most historical sites, representing 25.3% and 21.5% by weight (Figure 30), whereas fine sand ( $\geq 0.125$  mm –  $< 0.25$  mm) was the second-most dominant substrate class at the remaining four historical sampling sites (Figure 30). At historically occupied sites, the percent composition of sand and fine gravel ranged between 73.8–98.8% by weight, with a mean of 89.8%. At the non-historical, targeted sampling sites, the dominant substrate classes were fine sand and medium sand where the percent composition of sand and fine gravel ranged from 91.5% to 98% by weight, with a mean of 95.3% (Barnucz et al. 2022).

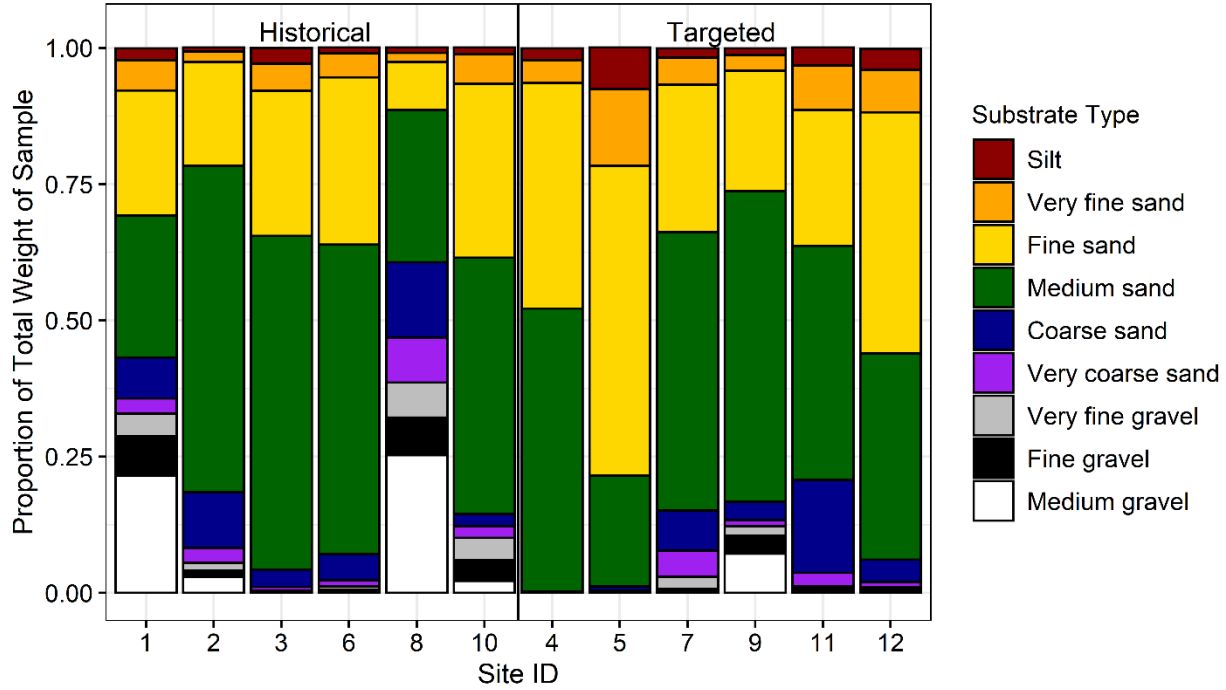


Figure 30. Substrate composition as the proportion of total weight of sample from historical (left) and targeted (right) sites in Big Otter Creek, Ontario (2020). Substrate categories: silt (< 0.063 mm; red), very fine sand (0.063–0.125 mm; orange), fine sand (0.125–0.25 mm; yellow), medium sand (0.25–0.50 mm; green), coarse sand (0.5–1.0 mm; blue), very coarse sand (1.0–2.0 mm; purple), very fine gravel (2.0–4.0 mm; grey), fine gravel (4.0–8.0 mm; black), and medium gravel ( $\geq 8$  mm; white). Recreated from Barnucz et al. (2022).

Substrate sampling was performed in the Grand River to allow comparisons of the proportional weight of silt (< 0.063 mm), sand and fine gravel (0.063–8.0 mm), and medium gravel ( $\geq 8$  mm) between the Grand River ( $n = 1$  site), historical sites in Big Otter Creek ( $n = 6$  sites), and targeted sites in Big Otter Creek ( $n = 6$  sites; Britney Firth, University of Waterloo, pers. comm.). Each site had five replicates. Similarity in substrate composition was evaluated using Horn's index of niche overlap ( $\hat{R}_0$ ):

$$\hat{R}_0 = \frac{\sum(\hat{p}_{ij} + \hat{p}_{ik}) \ln(\hat{p}_{ij} + \hat{p}_{ik}) - \sum \hat{p}_{ij} \ln(\hat{p}_{ij}) - \sum \hat{p}_{ik} \ln(\hat{p}_{ik})}{2 \times \ln(2)} \quad \text{Equation (24)}$$

where  $p_{ij}$  is the proportion of substrate  $i$  at location  $j$  and  $p_{ik}$  is the proportion of substrate  $i$  at location  $k$  (Krebs 1999). This is a recommended approach to reduce bias when working with small sample sizes (Smith and Zaret 1982, Krebs 1999). Substrate was considered ecologically similar when  $\hat{R}_0$  exceeded 0.60 (Zaret and Rand 1971, Wallace Jr. 1981).

Results of this analysis suggest that substrate composition was ecologically similar between sites in the Grand River and historical sites in Big Otter Creek ( $\hat{R}_0 = 0.86$ ), between the Grand River and targeted sites in Big Otter Creek ( $\hat{R}_0 = 0.75$ ), and between historical and targeted sites in Big Otter Creek ( $\hat{R}_0 = 0.97$ ). The proportion of silt substrate differed across locations with targeted sites in Big Otter Creek having more silt than historical sites. Sites in the Grand River had less proportional cover of sand and fine gravel substrate (mean =  $51.98 \pm 30.07$  SD) than historical ( $89.56 \pm 14.76$  SD) and targeted locations ( $95.36 \pm 5.42$  SD) in Big Otter Creek (Figure 31). The Grand River had more medium gravel ( $46.96 \pm 13.59$  SD) than historical ( $8.92$

$\pm 2.61$  SD) and targeted ( $1.37 \pm 0.97$  SD) sites in Big Otter Creek, and historical sites had more gravel than targeted sites.

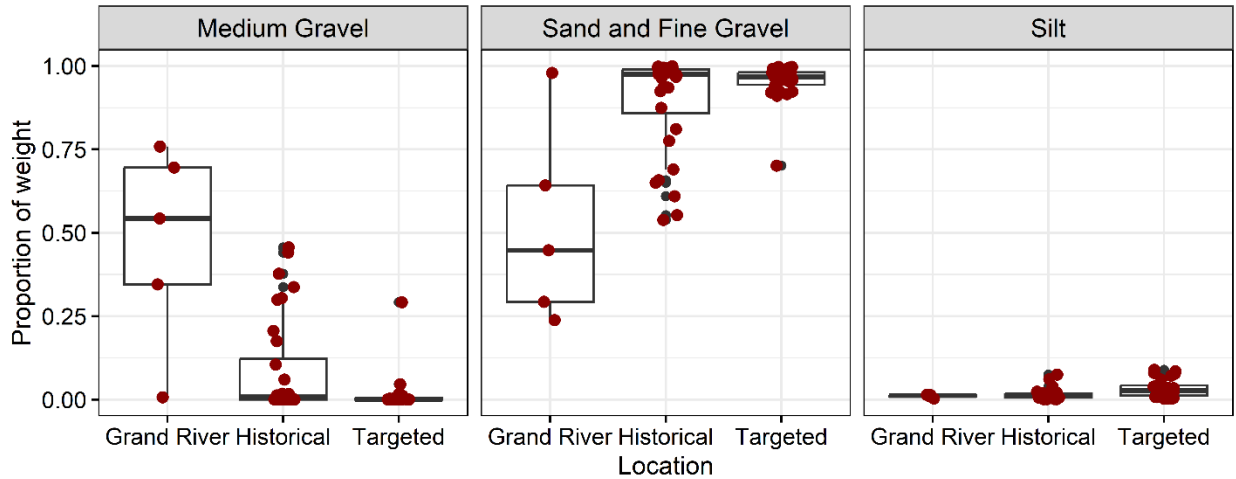


Figure 31. Relative proportion of total weight of medium gravel, sand and fine gravel, and silt at one site in the Grand River and 12 sites in Big Otter Creek (six historical and six targeted). Red dots indicate actual measurements and were jittered horizontally for visual purposes.

### Water clarity

Significant differences in average turbidity were observed across samples from Big Otter Creek, and sites in the Grand River and Thames River where Eastern Sand Darter was captured when either subset of data was considered (Table 18, Table 21). When all data were considered, the Grand River had the lowest average turbidity ( $14.12 \pm 38.23$  SD NTU), with 50% of measurements below 7.56 NTU. Alternatively, average water clarity measurements were significantly lower in Big Otter Creek than the Grand River when only considering data from Dextrase (2013;  $p < 0.001$ ). The sites where Eastern Sand Darter were captured in the Thames River showed a wide kernel density estimate and range of turbidity measurements when all data were considered (Figure 32) or when only considering data from Dextrase (2013; Figure 28). When considering all the data, there was approximately 68.25% and 15.03% overlap between kernel density estimates of turbidity in Big Otter Creek and sites where Eastern Sand Darter was captured in the Grand River and Thames River, respectively (Table 22). Similarly, there was a 45.17% overlap between kernel density estimates of water clarity measurements from Big Otter Creek and sites in the Grand River where Eastern Sand Darter was captured when considering the Dextrase (2013) data, with a greater overlap observed with measurements from the Thames River (58.07%; Table 22).

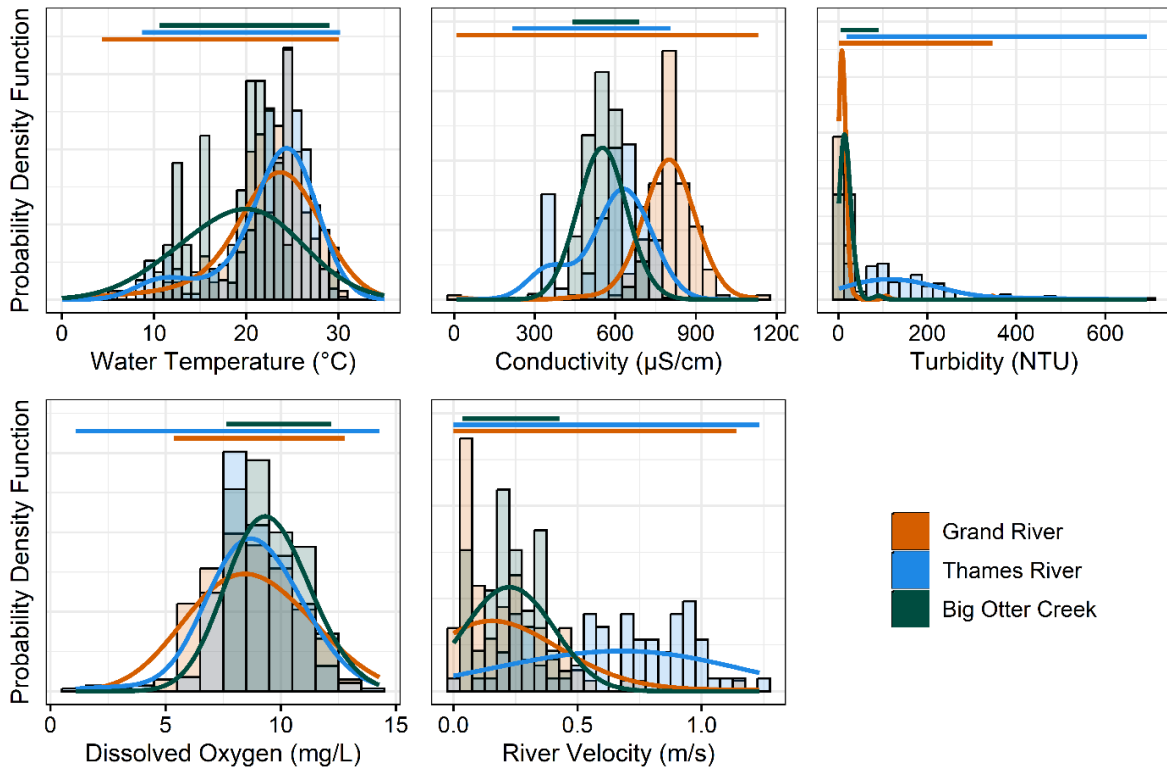


Figure 32. Histogram and kernel density plots for habitat measurements in Big Otter Creek (green) and at sites in the Grand River (orange) and Thames River (blue) where Eastern Sand Darter was captured. Sample sizes per variable per waterbody are presented in Table 20. Plots include all available data. Horizontal segments on the top of each plot indicate range of measured values per river system.

Table 22. Percent overlap in kernel density estimates for habitat variables in Big Otter Creek with measurements taken in the Grand and Thames rivers where Eastern Sand Darter was previously detected.

Data	Recipient Location	Habitat variable	Grand River	Thames River
All	Big Otter Creek	Water temperature (°C)	67.99%	64.54%
All	Big Otter Creek	Conductivity (µS/cm)	13.90%	59.21%
All	Big Otter Creek	Turbidity (NTU)	68.25%	15.03%
All	Big Otter Creek	Dissolved oxygen (mg/L)	73.16%	82.59%
All	Big Otter Creek	River velocity (m/s)	80.53%	34.30%
Dextrase	Big Otter Creek	Water temperature (°C)	65.74%	60.64%
Dextrase	Big Otter Creek	Conductivity (µS/cm)	0.06%	33.56%
Dextrase	Big Otter Creek	Water clarity (cm)	45.17%	58.07%
Dextrase	Big Otter Creek	Dissolved oxygen (mg/L)	51.53%	68.27%
Dextrase	Big Otter Creek	River velocity (m/s)	88.78%	88.61%

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### Other habitat characteristics

The variance of river velocity measurements was significantly different between Big Otter Creek and sites in the Grand and Thames rivers with Eastern Sand Darter captures when all data were considered (Table 20), but not when the data from Dextrase (2013) were considered independently (Table 18). There was a wider distribution of river velocity measurements in the Thames River than Big Otter Creek or the Grand River (Figure 32). Average river velocity measurements were significantly different among sites surveyed in Big Otter Creek, the Grand River, and the Thames River (Table 18; Table 21). However, no significant pairwise differences were observed with river velocity measurements recorded in Big Otter Creek based on either data subset. There was approximately 80.53% and 34.30% overlap between kernel density estimates of river velocity in Big Otter Creek and sites where Eastern Sand Darter was captured in the Grand River and Thames River, respectively, when all data were considered (Table 22). A similar pattern was observed in the Dextrase (2013) subset, where the kernel density estimates from Big Otter Creek overlapped by 88.78% and 88.61% with kernel density estimates from the Grand and Thames rivers, respectively (Table 22).

Significant differences in mean water temperature measurements were observed across samples from Big Otter Creek and sites where Eastern Sand Darter was captured in the Grand River and Thames River (Table 18; Table 21; Figure 28; Figure 32). On average and across both data subsets, water temperature measurements from Big Otter Creek were cooler than the Grand ( $p < 0.001$ ) and Thames ( $p < 0.001$ ) rivers, with no significant pairwise differences observed between the Grand and Thames rivers ( $p = 1.000$ ). When considering all the data, the Grand River had the largest range of water temperature measurements (4.37–30.10°C), followed by the Thames River (8.70–30.20°C) and Big Otter Creek (10.57–29.04°C; Figure 32). There was approximately 67.99% and 64.54% overlap in kernel density estimates of water temperature between Big Otter Creek and the Grand and Thames rivers, respectively (Table 22). The range of temperatures measured were similar in the Grand (15.5–27.7°C) and Thames (15.9–29.1°C) rivers when considering only the Dextrase (2013) data; a greater range of measurements was observed in these rivers than Big Otter Creek (18.4–22.8 °C; Figure 28). There was approximately 65.74% and 60.64% overlap in kernel density estimates of water temperature between Big Otter Creek and the Grand and Thames rivers, respectively (Table 22; Figure 28).

In addition to the point estimates of water temperature, daily time series data were available for the Grand River, Thames River, and Big Otter Creek from loggers deployed between 2019 and 2021 (Figure 33). A two-way ANOVA suggested a statistically significant interaction between the effects of month and river identity ( $F = 71,290$ ,  $p < 0.001$ ); Big Otter Creek had the lowest average stream temperature in seven months of the year relative to the Grand and Thames rivers (Figure 34).

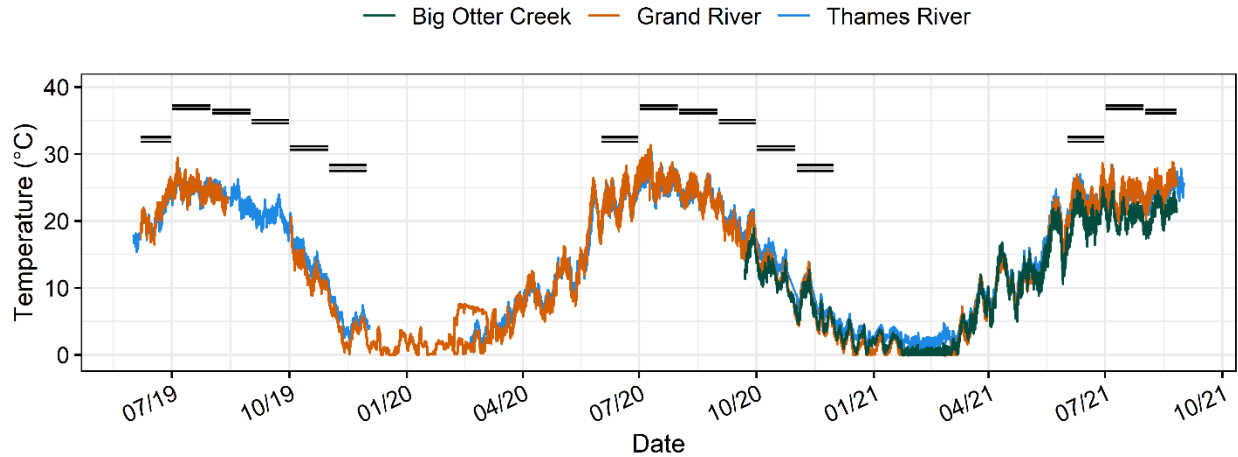


Figure 33. Daily mean water temperature measurements from the Grand River (orange; Cockshutt Bridge), Thames River (blue; upstream of Springbank Dam), and Big Otter Creek (green; downstream of Eden Road Bridge and off John Pound Road) between June 2019 and August 2021. Horizontal bars represent monthly (June–November) mean  $CT_{max} \pm 95\%$  confidence interval for Eastern Sand Darter from the Grand River (Firth et al. 2021a).

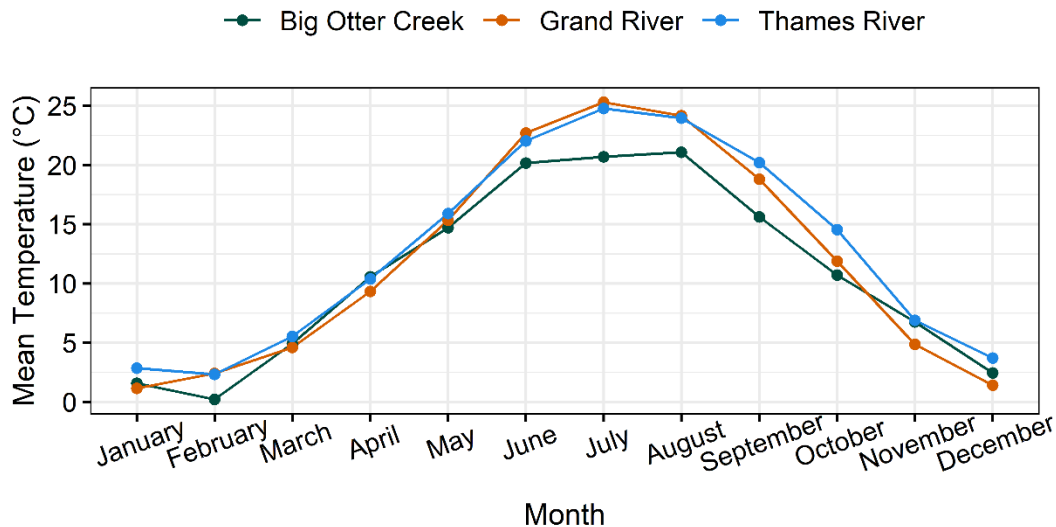


Figure 34. Mean monthly water temperature measurements from the Grand River (orange; Cockshutt Bridge), Thames River (blue; upstream of Springbank Dam), and Big Otter Creek (green; downstream of Eden Road Bridge and off John Pound Road) between June 2019 and August 2021.

The mean and variance of conductivity measurements were significantly different between Big Otter Creek and sites where Eastern Sand Darter was captured in the Grand River and Thames River (Table 17, Table 18, Table 20, Table 21). On average and across both data subsets, conductivity measurements in Big Otter Creek were the lowest and least variable when compared to sites in the Grand River or Thames River (Figure 28; Figure 32). Across all data, the Grand River had the greatest range of conductivity measurements (8.66–1,132.00  $\mu\text{S}/\text{cm}$ ), followed by Big Otter Creek (440.3–688.00  $\mu\text{S}/\text{cm}$ ) and the Thames River (217.00–806.20  $\mu\text{S}/\text{cm}$ ; Figure 32). There was approximately 13.90% overlap in the kernel density estimate of conductivity between Big Otter Creek and sites where Eastern Sand Darter was previously

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detected in the Grand River (Table 22). This is substantially lower than the 59.21% overlap observed between Big Otter Creek and the Thames River where Eastern Sand Darter was captured (Table 22). A similar pattern was observed when considering only the Dextrase (2013) data, where there was no overlap (0.06%) in the kernel density estimate of conductivity between Big Otter Creek and sites where Eastern Sand Darter was captured in the Grand River, with 33.56% overlap observed with the Thames River (Table 22; Figure 28). The Thames River had the greatest range of conductivity measurements when considering only the Dextrase (2013) data (471–720  $\mu\text{S}/\text{cm}$ ), with the Grand River (715–842  $\mu\text{S}/\text{cm}$ ) also having a greater range of measurements than Big Otter Creek (527–596  $\mu\text{S}/\text{cm}$ ; Figure 28).

The mean and variance of dissolved oxygen measurements differed across rivers (Table 17; Table 18; Table 20; Table 21), with Big Otter Creek having significantly higher mean dissolved oxygen with less variance than measurements recorded in the Grand River ( $p = 0.006$ ) or Thames River ( $p = 0.005$ ). Nevertheless, there was 73.16% and 82.59% overlap in kernel density estimates between Big Otter Creek and the Grand and Thames rivers, respectively (Table 22). Note that significant differences in mean dissolved oxygen measurements were observed in the Dextrase (2013) data (Table 18) but reflected a significantly lower average measurement in the Ausable River than the other rivers being considered. The range of measurements and kernel density estimate of dissolved oxygen measurements in Big Otter Creek was slightly narrower than the Grand River and Thames River sites when considering all available data (Figure 32) or when only considering the data collected in Dextrase (2013; Figure 28).

#### **Targeted dissolved oxygen sampling**

In addition to evaluating substrate composition at targeted and historically occupied sites in Big Otter Creek, dissolved oxygen was measured to allow comparisons with sites occupied by Eastern Sand Darter in the Grand River (Barnucz et al. 2022). Specifically, differences in dissolved oxygen 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 dissolved oxygen between the surface and 1 cm into the substrate was calculated rather than directly comparing dissolved oxygen measurements. Analysis of variance was used to test for differences in dissolved oxygen between Big Otter Creek targeted sites, Big Otter Creek historical sites, and the Grand River (Britney Firth, University of Waterloo, pers. comm.).

The difference in dissolved oxygen was significantly different between collection locations ( $F_{2,140} = 7.99$ ,  $p < 0.001$ ); targeted sites in Big Otter Creek had a greater difference in mean dissolved oxygen between surface and sediment dissolved oxygen (9.69 mg/L  $\pm$  2.76 SD) compared to historical sites in Big Otter Creek (7.44 mg/L  $\pm$  3.59 SD) and the Grand River (7.44 mg/L  $\pm$  2.56 SD). This suggests that there is less dissolved oxygen available 1 cm into the substrate at targeted sites in Big Otter Creek relative to the other two locations, but historical sites are similar.

#### **Question 12: Connectivity in Big Otter Creek for supporting all life-stages**

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 anticipated Eastern Sand Darter translocations. The second dam is located on the main stem at Otterville, Ontario (Loomer 2011), marking the upstream boundary of potential Eastern Sand Darter translocations. As well, small dams have been constructed on virtually all tributaries of Big Otter Creek to store water for irrigation (Loomer 2011). Overall, there are fewer barriers in the mainstem of Big Otter Creek than the Grand River. It is unknown whether or how the present-day dams would impair connectivity of a reintroduced population.

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## Biotic considerations

The community in the recipient ecosystem can influence the probability of achieving a successful reintroduction. Understanding the biotic interactions that affect Eastern Sand Darter and the niche that the species fills relative to co-occurring species can help determine whether recipient sites meet biotic requirements.

### Question 13: Availability of food resources in the Ausable River

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 broadly described as consisting of larval Chironomidae, Ostracoda, Cladocera, Ephemeroptera, and Oligochaeta based on stomach contents analysis (Turner 1921, Smith 1979, Spreitzer 1979, Facey 1998, Finch 2009). A quantitative assessment using a combined approach of stomach content and stable isotope analyses was performed on Eastern Sand Darter from the Thames River (Burbank et al. 2019). The analyses focused on four groups:

1. Chironomidae,
2. Ostracoda,
3. Cladocera, and
4. Other (including Ephemeroptera and Oligochaeta).

Across these groups, approximately 51% of stomach contents consisted of Chironomidae, 21% Ostracoda, 4% Cladocera, and 9% Other; approximately 15% of the stomach contents consisted of sediment (Burbank et al. 2019). Stable isotopes suggested that approximately 33% [14–65% (95% CI)] of Eastern Sand Darter diet consisted of Ephemeroptera, 29% Oligochaeta (0–44%), 19% Chironomidae (9–31%), 17% Ostracoda (4–35%), and 1% Cladocera (0–2%). The isotopic niche size of Eastern Sand Darter was determined to be more narrow than co-occurring species [Blackside Darter (*Percina maculata*), Johnny Darter (*Etheostoma nigrum*)], with the greatest likelihood of overlap occurring with these two species.

Determining whether sufficient food resources would exist to support a reintroduced population would require multi-year, seasonal sampling in the recipient ecosystem. The best information would come from a seasonal monitoring program that sampled for benthic macroinvertebrates and fishes documenting relative abundance of captures, alongside the use to stomach-content examinations and stable isotope analyses to investigate the diet of the resident benthic fish community.

Fisheries and Oceans Canada has not performed benthic macroinvertebrate sampling in the Ausable River; however, a benthic data set was provided by the Ausable Bayfield Conservation Authority (Kari Jean, Ausable Bayfield Conservation Authority, pers. comm.). Data from three sites within the area being considered as potential reintroduction habitat were available where sampling occurred multiple times between 2017 and 2022, all during the month of October. Included were sites near Exeter, Ontario in the upstream section of the Ausable River, and sites near Arkona and Springbank, Ontario in the middle section of the Ausable River. Benthic samples were collected using a three-minute walking-kick technique with a D-frame net (250 µm mesh; ABCA 2009). In the laboratory, samples were sub-sampled using a Marchant box, with individual cells randomly selected for invertebrate identification. Across the three surveyed sites, 17 orders, 37 families, and at least 21 genera of benthic macroinvertebrates were identified (Table A2). Although only one genus from the Chironomidae family was identified (*Cricotopus*), 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, characterized

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by at least five genera. Moreover, oligochaetes from the Naididae family were detected at two of the three sites, including the most upstream site in Exeter.

#### **Question 14: Availability of food resources in Big Otter Creek**

In October 2022, benthic macroinvertebrates were sampled from six sites in Big Otter Creek to better understand the diversity of prey for Eastern Sand Darter in the system (Nicholas Mandrak, University of Toronto Scarborough, pers. comm.). Of the six sites, four were historically occupied locations while the other two were considered potentially suitable for Eastern Sand Darter based on substrate characteristics (i.e., targeted; Barnucz et al. 2022). A kick-and-sweep approach was used to sample benthic macroinvertebrates following the Ontario Benthos Bionetwork methods (Jones et al. 2007). Three sample locations along a single transect across the stream perpendicular to the bank were sampled at each site (left bank, middle, right bank), with specimens grouped into one jar per site. Benthic macroinvertebrates were sampled at each site until approximately 300 specimens were collected. Once this threshold was reached, specimens were transferred with a paintbrush into 500-micron sieves, rinsed in water, and stored for identification in the laboratory.

In the laboratory, each sample jar was emptied onto a 30 x 30 cm plastic tray for sorting. Larger, visible invertebrates (e.g., Odonata) were removed first, followed by the use of a saline solution to sort smaller individuals (1–2 mm in size). After approximately 300 individuals were sorted (per site), specimens were transferred into a Marchant box for subsampling. Specimens were identified to various taxonomic levels using a dissecting microscope (Jones et al. 2007, Stanfield 2017). All invertebrates were further identified to the genus or species level using online and physical resources (Clench and Fuller 1965, Johnson 1974, Alstad 1980, Voshell and Wright 2002, Anderson 2009, Thorp and Rogers 2011, Xu et al. 2015).

Across the six surveyed sites, 12 orders, 20 families, and 32 genera were identified (Table A3). For most of the taxa (31/46 = 67.4%), individuals were only detected at a single site. *Hesperocorixa vulgaris* was identified as the most abundant taxa ( $n = 323$  individuals), while *Choronomus* sp. was present at the most sites ( $n = 5$  sites). *Chematopsyche analis*, *Hydropsyche carolina*, and *Choronomus* sp. were the second, third, and fourth most abundant taxa identified (Table A3). 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); however, no Oligochaeta were detected.

#### **Questions 15–16: Influence of co-occurring species on reintroduction**

The relative abundance of fish species in freshwater ecosystems is dynamic, fluctuating in space and time with patterns of births, deaths, immigration, and emigration. During and following a species extirpation, the fish community composition continues to shift in relative abundance over space and time. In some cases, secondary extirpations may occur (Brodie et al. 2014), further altering the local fish community structure. In either case, the fish community structure at the recipient location is unlikely to match the structure when the species was historically present. This can lead to scenarios where the resident fish community ‘resists’ reintroduction efforts, preventing population growth or sustained and stable life cycles of the reintroduced species by means of negative interactions (e.g., predation; Tielke et al. 2020). Predicting whether the community is ecologically closed to reintroduction is difficult in the simplest of food-web models (Tielke et al. 2020) and has yet to be considered for wild freshwater assemblages.

There is no research that suggests Eastern Sand Darter has an obligate, facultative, or parasitic species dependency that would limit the ability to be successfully reintroduced. Eastern Sand

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Darter was recently identified as a host species for Round Hickorynut (*Obovaria subrotunda*) in Kentucky, United States (Shepard et al. 2021). Round Hickorynut is a small freshwater mussel listed as Endangered under SARA due to significant declines over the last 30 years, including extirpations from the Grand and Thames rivers (DFO 2013, 2022). There are no historical records of Round Hickorynut in the Ausable River or Big Otter Creek.

No studies have quantified direct interactions between Eastern Sand Darter and co-occurring species. Similar to evaluating habitat requirements, quantifying biotic interactions generally requires manipulation or experimentation. Instead, researchers often investigate biotic associations, or observed species co-occurrence patterns (Peres-Neto 2004), including for Eastern Sand Darter (Lamothe et al. 2019, 2019c). For example, Eastern Sand Darter has been identified as positively associated with 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. 2019b). The mechanisms behind these relationships are unclear but are likely related to habitat preferences rather than direct interactions. This would align with previous research that has demonstrated the elevated role of species-habitat relationships driving species co-occurrence patterns in stream fishes, with biotic interactions having a lesser impact (Peres-Neto 2004).

Species lists were generated to compare fish communities between potential source and recipient locations (Table A4). Note that the data used to generate these lists differed significantly by frequency of sampling, gear type, and the spatial extent of sampling, and therefore caution is warranted around interpretation of differences in the community. The Thames River (within the area of Eastern Sand Darter critical habitat) has the most species documented relative to the three other focal systems ( $n = 74$  species detected). The Grand River has supported at least 61 species over the period of sampling, 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 (Table A4). Five species were detected in the Thames River that were not recorded in any of the other systems being considered, with no unique species captured in the Grand River. There were seven unique species detections in the Ausable River and one in Big Otter Creek (Table A4); 77.3% of the species captured in the Ausable River were shared with the Grand River, with 86.4% shared with the Thames River. In comparison, 88.9% of the species captured in Big Otter Creek were shared with the Grand River, with 94.4% being shared with the Thames River.

Eastern Sand Darter is assumed to be vulnerable to predation by piscivores and competes with other benthic species for resources. Potential predators and competitors of Eastern Sand Darter were previously identified based on feeding habits and habitat preferences (Dextrase 2013); piscivores that occupied shallow (< 1 m) sandy pools and runs were considered as predators, and insectivores and invertivores that preferred the same habitat were characterized as potential competitors (Dextrase 2013). Species not detected in Dextrase (2013) were characterized accordingly based on regional publications (Portt et al. 1999, Coker et al. 2001, Holm et al. 2021). In total, 21 predators and 56 competitors of Eastern Sand Darter were identified across the potential source and recipient waterbodies (Table A4). The Ausable River and Thames River both support at least 17 presumed predators of Eastern Sand Darter, while the Grand River supports 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 (Table A4).

The invasion of Round Goby into the Great Lakes region occurred decades after the last records of Eastern Sand Darter in the Ausable River or Big Otter Creek. At present, Round Goby has been documented as far upstream as Tillsonburg, Ontario within Big Otter Creek (Barnucz et al. 2020) and upstream to Sylvan, Ontario in the Ausable River (Figure 13). The

effects of Round Goby on Eastern Sand Darter are discussed under the Threat Considerations section.

### Threat considerations

Reintroduction planning must consider the potential effects of threats on source populations and recipient ecosystems (Robinson et al. 2020). The primary reason for failing to re-establish a population by reintroduction is the failure to address threats that originally caused the extirpation (Armstrong and Seddon 2008, Cochran-Biederman et al. 2014, Bubac et al. 2019). There are two threats that are most often implicated for impacting Eastern Sand Darter survival, growth, and (or) reproduction in southwestern Ontario: agricultural activities and invasive species (DFO 2012; Figure 35). Below, the anticipated effects of agriculture and invasive species on the probability of re-establishing a population in potential recipient locations relative to the effects of threats on potential source populations are described.

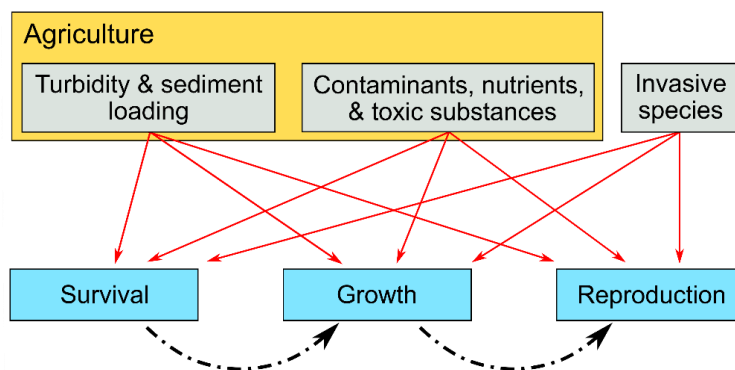


Figure 35. Conceptual diagram representing the negative effects of the most relevant threats (grey) to Eastern Sand Darter growth, survival, and reproduction in southwestern Ontario. Red arrows indicate anticipated effects of the threat on the particular life-history process. Black arrows indicate the relationship between survival, growth, and reproduction such that impacts to survival can lead to changes in reproduction.

#### Question 17: Agricultural activities in the Ausable River watershed

Increased turbidity and sediment loading, along with the introduction of contaminants, nutrients, and toxic substrates, are commonly identified as consequences of agricultural practices on freshwater ecosystems (Figure 35). 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). Negative impacts of turbidity and sediment loading include the smothering of eggs, reductions in the number and quality of suitable spawning areas leading to decreased egg survival, decreases or reduced growth rates of juveniles, reduced oxygen within the substrate, and (or) adversely affecting prey abundance (Berkman and Rabeni 1987, Holm and Mandrak 1996, Drake et al. 2008, Finch 2009, Kemp et al. 2011, 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. In the Grand River watershed, approximately 61% of the land is used for agricultural production, with approximately 14% being urban and built-up areas (including roads), and 15% being forest cover (GRCA 2020). There were 5,641 farms in 2016, with crop production being the primary form of agriculture in the Grand River watershed (> 80%; GRCA 2020). Previous research has identified that nutrient inputs from the Grand River watershed contributes approximately 5% of the total annual phosphorus loading to Lake Erie (40% of the annual total to the eastern basin), with

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manure application being the largest source of phosphorus export (IJC 2014, Scavia et al. 2014, Shaker 2014, Hanief and Laursen 2019). Similarly, the Thames River watershed is composed of approximately 80.0% agricultural land, with 7.8% being urban and built-up areas (including roads), and 5.1% being forest cover (Nürnberg and LaZerte 2015). This watershed continues to have poor scores on watershed report cards, generally not meeting provincial water quality objectives for phosphorus or *Escherichia coli* (LTVCA 2018).

Similar to the Grand and Thames river watersheds, the Ausable River watershed is dominated by agriculture (> 70% land cover; ABCA 2015). The 2018 Watershed Report Card suggested fair surface water quality in the Ausable River watershed based on measurements of total phosphorus, concentrations of *E. coli*, and surveys of benthic invertebrates (Coleman et al. 2018). Water-quality measurements (alkalinity, conductivity, pH, sodium, and turbidity) have been recorded at several locations within the Ausable River watershed on a monthly basis for over two decades, including three sites within the area being considered for reintroduction efforts (Sylvan, Springbank, Exeter; Figure 36). Generally, water-quality metrics have remained consistent over time, with a slight decline in pH and increase in conductivity observed across sites. Turbidity measurements were higher in areas downstream of Exeter, with sodium and conductivity measurements being highest at the Exeter site (Figure 36).

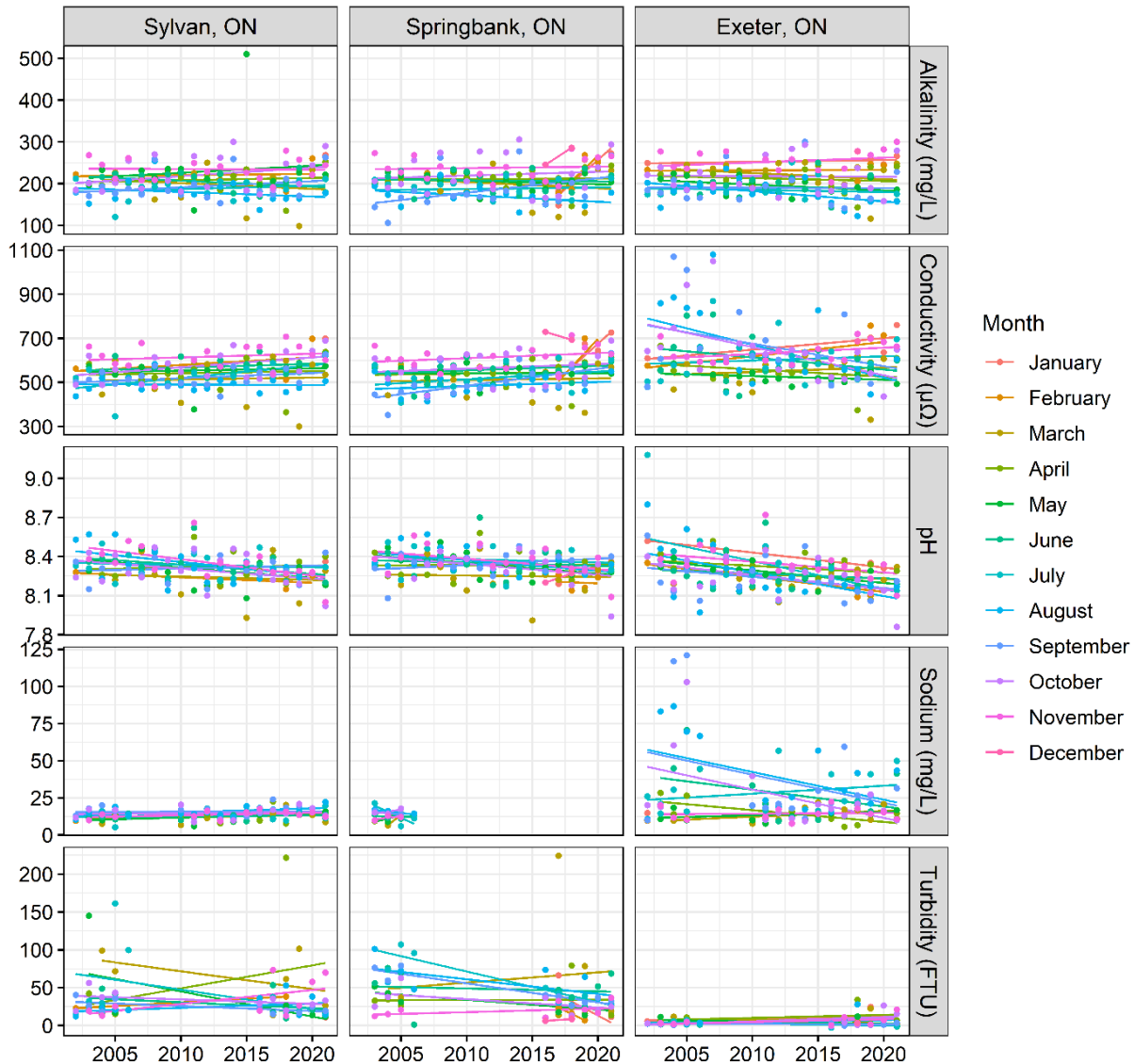


Figure 36. Alkalinity (mg/L), conductivity ( $\mu\Omega$ ), pH, sodium (mg/L), and turbidity (Formazine Turbidity Unit; FTU) measurements (y-axis) by month (colour) across years (x-axis) from sites in Sylvan, Springbank, and Exeter, ON.

### Question 18: Round Goby in the Ausable River watershed

Round Goby is identified as a threat to the persistence of Eastern Sand Darter in Ontario (DFO 2012, 2018). Determining whether Round Goby would prevent the establishment of an Eastern Sand Darter population in the Ausable River requires an understanding of Round Goby abundance and distribution in the system, the relative effects of Round Goby on fitness or physiology, along with experiments to understand the probability of establishment with respect to Round Goby density and other important variables (e.g., habitat conditions). In the absence of experimental data, knowledge of the distribution of Round Goby within areas of Eastern Sand Darter occupancy and information gained from the literature on the effects of Round Goby on life-history processes of benthic fishes can inform predictions on the potential effects on Eastern Sand Darter establishment.

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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 farther upstream in Arkona, Ontario (Figure 13). However, sampling downstream of Ailsa Craig has not been performed since 2017. Further sampling is required to determine the present-day upstream boundary of Round Goby distribution in the Ausable River.

Round Goby and Eastern Sand Darter have co-occurred in the Grand and Thames rivers for more than a decade. Records of Round Goby in the Grand River span from the downstream limit of Eastern Sand Darter critical habitat, upstream to the Brantford Landfill (Figure 4). Within this stretch, there are 37 records where Eastern Sand Darter was captured at the same site with Round Goby (2009, 2010, 2013, 2016). In the Thames River, records of Round Goby stretch from the downstream boundary of Eastern Sand Darter critical habitat to the Dutton-Dunwich Conservation Area. Similarly, there are 22 records from the Thames River where Eastern Sand Darter was captured with Round Goby (2006, 2015, 2016, 2022). All of the observed co-occurrences in the Grand and Thames rivers were captured in deeper water using a trawl, which could suggest that areas preferred by Eastern Sand Darter and conducive to sampling using a seine (e.g., stream depth > 1.2 m) in the Grand and Thames rivers are less preferred by Round Goby.

Round Goby can have negative effects on the survival, growth, and (or) reproduction of Eastern Sand Darter (Figure 35), however, these effects have not been studied in full for the species. Competition and egg predation are the primary mechanisms resulting in negative consequences to benthic fish species (Bergstrom and Mensinger 2009, Kornis et al. 2012, Morissette et al. 2018). Such negative interactions could be observed through dietary overlap and shifts in feeding strategies (Firth et al. 2021b) or by forcing Eastern Sand Darter into suboptimal habitats (e.g., van Snik Gray et al. 2005, Leino and Mensinger 2017), reducing the probability of survival, growth, and (or) reproduction (Figure 35). Sampling in the Ausable River and Big Otter Creek do not suggest significant negative interactions between Round Goby and co-occurring Percidae species (McAllister et al. 2022), though some evidence exists of changes in the isotopic niche of benthic species (including Blackside Darter, a Percidae species) when Round Goby is present (McAllister 2022).

Diet overlap has been demonstrated between Round Goby and other benthic fish species in invaded regions (Rakauskas et al. 2013, Ustups et al. 2016, Uzunova and Dashinov 2022), including Eastern Sand Darter (Firth et al. 2021b). Gut-content analysis was performed on nine benthic species (5–78 individuals per species) from the Sydenham River, Ontario captured before and after the invasion of Round Goby (Poos et al. 2008, Firth et al. 2021b). Prior to the invasion, there was significant dietary overlap (Morista's  $\hat{C}$ ) between Eastern Sand Darter and Brindled Madtom (*Noturus miurus*) and between Eastern Sand Darter and Johnny Darter (Firth et al. 2021b). Following invasion, Eastern Sand Darter diet overlapped with Brindled Madtom and Johnny Darter, along with Greenside Darter (*Etheostoma blennioides*) and Round Goby (Firth et al. 2021b). A general pattern of niche convergence was observed across species after the invasion of Round Goby, where a greater frequency of pairwise overlaps in diet were observed among the fish community alongside a shift toward a specialist diet (Chironomidae; Firth et al. 2021b).

Egg predation by Round Goby has direct negative effects on recruitment of fishes and has been observed for several species (Leblanc et al. 2020, Lutz et al. 2020). For example, predation of Common Nase (*Chondrostoma nasus*) eggs by Round Goby in Switzerland was determined using molecular techniques (Lutz et al. 2020). However, there have not been any direct observations of egg predation on Eastern Sand Darter eggs.

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Decline in the abundance of Eastern Sand Darter has been at least partially attributed to the invasion of Round Goby (Stauffer Jr. et al. 2016), including in the Grand River, Ontario (Raab et al. 2018). Sampling in the Sydenham River, a tributary that contains an Eastern Sand Darter population in poor condition (Table 1), supports this conclusion (Craig Paterson, St. Clair Region Conservation Authority, pers. comm.). The Sydenham River has experienced a rapid Round Goby expansion over the last decade. Fish community data were collected from 10 sites in 2016, 2020, 2021, and 2022 using backpack electrofishing and seines. Fifty-six Eastern Sand Darter were captured in 2016, one individual was captured in 2020, and zero were captured in 2021 and 2022. In comparison, 358 Round Goby were captured in 2016, 196 in 2020, 161 in 2021, and 236 in 2022. Note that caution is warranted in the interpretation of these data as they consist of four snapshots in time across a relatively small spatial scale and may not be fully representative of the true state of the fish community. Nevertheless, Eastern Sand Darter was more abundant at these sites prior to 2016 based on historical accounts (Poos et al. 2008), suggesting a declining trend.

### **Questions 19 and 22: Disease in the Ausable River and Big Otter Creek**

Disease could prevent the establishment of individuals at the recipient location, 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). Overall, there is limited knowledge about the impacts of pathogens and parasites on Eastern Sand Darter and for most fishes that it co-occurs with. The National Code on Introductions and Transfers of Aquatic Organisms identifies that Fisheries and Oceans Canada along with the province of Ontario is responsible for managing disease risks pertaining to the intentional movement of aquatic organisms that fall outside the scope of the National Aquatic Animal Health Laboratory System (Government of Canada 2017). An assessment has yet to be performed on the risk of disease transfer when performing reintroductions of Eastern Sand Darter.

### **Question 20: Agricultural activities in the Big Otter Creek sub-watershed**

Like the Grand, Lower Thames, and Ausable river watersheds, the Big Otter Creek sub-watershed is dominated by agriculture (within Long Point Region watershed). Due to historical practices around tobacco cultivation, freshwater ecosystems were left severely impacted by sediment loads, excessive turbidity, and changes in water chemistry (DFO 2012). Based on watershed report cards (Coleman et al. 2018, LPRCA 2018), a similar concentration of *E. coli* (101-300 colony-forming units per 100 mL of water) and total phosphorus levels (total phosphorus range: 0.031–0.060 mg/L) affect surface water quality in the Big Otter Creek sub-watershed and the Ausable River watershed. However, total forest cover is higher in the Big Otter Creek watershed, with approximately 15.1–25.0% total forest cover and a percent forested riparian zone of 27.6–42.5% (LPRCA 2018). Similar seasonal patterns in water chemistry from the Ausable River (Figure 36) have been observed in Big Otter Creek (2007–2008), where turbidity, suspended solids, total phosphorus, residual phosphorus, and phosphate concentrations were higher and more variable during the winter than the spring, summer, or fall months, matching the flow regime in the system (Loomer 2011).

### **Question 21: Round Goby in the Big Otter Creek sub-watershed**

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 system; crews only captured Common Shiner (*Luxilus cornutus*) and Johnny Darter in greater abundance than Round Goby, with the most prevalent species being Common Shiner (detected at 84.62% of sites), Creek Chub (*Semotilus atromaculatus*; 75%), Johnny Darter (67.31%), Round Goby (63.46%), and White Sucker (*Catostomus commersonii*; 59.62%; Barnucz et al. 2020). 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 in the system (Barnucz et al. 2020). As of 2018, Round Goby had not reached Rock Mills, Ontario (Barnucz et al. 2020).

### **3. INFORMATION IN SUPPORT OF ESTIMATING THE PROBABILITY OF UNINTENDED CONSEQUENCES RESULTING FROM EASTERN SAND DARTER REINTRODUCTION EFFORTS**

The next 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. For the context of Eastern Sand Darter reintroduction, this includes population, community, and ecosystem related risks and consequences at the source and recipient locations. Similar to Step 2, the goal of Step 3 is to score the probability of incurring unintended consequences due to the removal and (or) establishment of individuals in the source and recipient locations (Table 23). Below, eight 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 use in a modified-Delphi process, where experts provide answers along with a measure of uncertainty based on their interpretation and evaluation of the question.

*Table 23. Ecological risk considerations for the focal taxa and other ecosystem components in source and recipient habitats of proposed reintroductions.*

<b>No.</b>	<b>Question</b>
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?

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No.	Question
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?
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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### **QUESTION 23: INBREEDING DEPRESSION IN THE SOURCE POPULATION**

Removing a significant number of individuals from a population can cause elevated rates of mating between closely related individuals, threatening inbreeding depression and possibly reducing long-term adaptive potential. 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). Inbreeding coefficients were calculated for the Thames and Grand River populations, with two sites in the upper stretch of the Thames River showing statistically significant inbreeding, albeit with relatively low coefficients ( $F_{IS} < 0.1$ ; Ginson et al. 2015). However, this study did not test for relatedness between individuals and therefore these results could be influenced by sampling design.

### **QUESTION 24: INBREEDING DEPRESSION IN THE RECIPIENT POPULATION**

Introducing too few individuals to a recipient location could lead to inbreeding depression in the reintroduced population, potentially resulting in reduced long-term adaptive potential. Ensuring that translocated individuals are sampled from across the within-river range of the source population can reduce the potential for inbreeding depression in the recipient location. Moreover, the likelihood of inbreeding depression is reduced when more individuals are reintroduced. 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 recipient location is likely to be of minimal concern for a reintroduced Eastern Sand Darter population.

### **QUESTION 25: FOUNDER EFFECT IN THE RECIPIENT LOCATION**

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 (Chen et al. 2012) and captive breeding programs (Saavedra and Guerra 1996) and can be of concern for reintroduction efforts when the number of individuals introduced is low (e.g., 30 individuals; Jamieson 2011). For example, 457 Fountain Darter (*Etheostoma fonticola*) were reintroduced in the early 1970s to

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the Comal River, Texas, USA using individuals collected from the San Marcos River, Texas; recent genetic studies suggest that the species underwent a founder effect following this reintroduction, with approximately 49 individuals actually contributing as founding individuals (Olsen et al. 2016). Nevertheless, the population has grown to over 150,000 fish suggesting a successful conservation outcome (Olsen et al. 2016).

Multiple small introductions of randomly selected (or stratified random selection), unrelated individuals with sufficient genetic diversity from the source to recipient location will significantly 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 river distribution. While also reducing the probability of experiencing a founder effect, sourcing individuals across its entire river range reduces the impact of harm at the site scale.

### **QUESTION 26: OUTBREEDING DEPRESSION AS A RESULT OF MIXING GRAND AND THAMES RIVER POPULATIONS**

Outbreeding depression is defined as a reduction in fitness caused by the crossing of genetically distinct populations. Different mechanisms can lead to outbreeding depression including chromosomal differences between populations, adaptive differentiation between locations, genetic drift, and population bottlenecks (Frankham et al. 2011). 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 the source population. In turn, reduced fitness of the reintroduced population would make it more vulnerable to collapse in the face of natural and anthropogenic disturbances. The probability of experiencing outbreeding depression is highest when two populations have fixed chromosomal differences, have not exchanged genes in the last 500 years, or occupy different habitats (Frankham et al. 2011).

Outbreeding depression has been observed in freshwater fish communities as a result of human-mediated movement of individuals. For example, reintroduction of Slimy Sculpin (*Cottus cognatus*) was performed in the tributaries of the upper Mississippi River (Minnesota, USA) using 2–3 local source populations from separate sub-drainages approximately 40 river km apart. Molecular analysis suggested that second-generation hybrids had reduced length, weight, and growth rates (fitness surrogates) than progeny from pure individuals (Huff et al. 2011). In experimental trials using Largemouth Bass (*Micropterus salmoides*) from Illinois and Wisconsin, outbreeding depression was observed in competitive breeding trials along with a greater susceptibility of mortality of hybrid individuals to Largemouth Bass ranavirus (Goldberg et al. 2005). However, other studies have demonstrated contrary results, where the use of multiple source populations leads to an advantage for hybrid progeny (i.e., heterosis). For example, two source populations were used for reintroducing Macquarie Perch (*Macquaria australasica*) to the Ovens River in eastern Australia; hybrid progeny (Yara x Dartmouth) had a greater survival rate than pure individuals from the Dartmouth line (Lutz et al. 2021). Similarly, no differences in survival or fitness-related surrogates were observed in the first generation of individuals bred from distinct Atlantic Salmon (*Salmo salar*) populations (Audet et al. 2017). Nevertheless, evolutionary theory suggests that a source population with shared genetic ancestry and (or) an individual population that occupies similar environmental conditions would be considered a lower risk than the mixing of multiple source populations.

### **QUESTION 27: INTERSPECIFIC HYBRIDIZATION IN THE RECIPIENT LOCATION**

Interspecific hybridization can lead to several outcomes that would not benefit the conservation of southwestern Ontario Eastern Sand Darter. With respect to conservation, interspecific hybridization can lead to the loss of unique genetic variation in pure populations. As phylogenetic distance between darter species increases, the frequency of interspecific

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hybridization decreases (Keck and Near 2009). Eastern Sand Darter comes from a monophyletic group indicating that it has a single ancestor that differs from most other darter species (e.g., *Etheostoma*; 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.

### **QUESTION 28: INTRODUCING DISEASE TO THE RECIPIENT LOCATION**

Translocating individuals from one location to another can lead to the movement and potential impacts of pathogens and parasites on fishes at the recipient location. Most areas in source and recipient locations are subject to migratory fish movements between the tributaries and Lake Erie, and therefore its expected that disease prevalence may be similar between source and recipient locations. However, as described for Questions 19 and 22, there is limited knowledge about the impacts of pathogens and parasites on Eastern Sand Darter and co-occurring species. An assessment has yet to be performed on the risk of disease transfer for Eastern Sand Darter reintroductions.

### **QUESTION 29: TRANSFORMATIVE CHANGES IN SOURCE LOCATIONS**

The removal of fishes from an ecosystem can lead to significant changes in fish community dynamics by making food or habitat 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 the risk that other species may capitalize on the available resources. In such a scenario, constraints on the growth of other species may be removed or 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. Given the removal of less than 2,000 individuals per year, its anticipated that the probability of transformative change is similarly low. Ultimately, the magnitude of effects that removals may have on community dynamics would require experimental trials and monitoring of fish abundance and quantification of, for example, shifts in food webs over time.

### **QUESTION 30: TRANSFORMATIVE CHANGES IN RECIPIENT LOCATIONS**

The invasion literature has many examples where the movement of a species to previously unoccupied areas cause transformative changes to the ecosystem within and beyond the location of introduction. 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 transformative changes vary based on the species and area that it invades. For example, benthic foraging by Common Carp (*Cyprinus carpio*) can transform aquatic ecosystems by destroying aquatic macrophytes and decreasing water quality in invaded areas (Weber and Brown 2009). However, Eastern Sand Darter has not had an opportunity to spread to areas outside of its historical range and lacks the characteristics of common invasive fishes (Olden et al. 2011, Karasov-Olson et al. 2021). Specifically, Eastern Sand Darter has a restricted distribution, is in low abundance, has a low tolerance to stressors, and a low rate of dispersal. Moreover, Eastern Sand Darter is believed to have previously occupied the candidate recipient locations. Ultimately, the probability of significant changes to the recipient community will be, in part, influenced by the ecological naivety of the source community to the translocated species (Lamothe et al. 2023).

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## APPLICATION

The information presented in this document was used to inform a Delphi expert judgement process during the CSAS meeting. The results of the Delphi process informed the science advise on the ecological benefits, risks, and feasibility of Eastern Sand Darter reintroductions. Results of the Delphi process have been published in the Science Advisory Report (DFO 2025), with discussions documented in the Proceedings (Colm et al. 2025).

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## APPENDIX I – SUMMARY OF DATA RECORDS

*Table A1. Sources of habitat and fish sampling data for Eastern Sand Darter records from the Ausable River, Big Otter Creek (downstream of Otterville), Catfish Creek, Grand River, and Thames River. Data for the Ausable River include sampling efforts upstream of The Cut and downstream of Dashwood Rd., Exeter, Ontario. Data for Big Otter Creek include sampling efforts downstream of Otterville, Ontario and upstream of Vienna, Ontario. Data from the Thames and Grand rivers include only areas within Eastern Sand Darter critical habitat. Included is information on year, number of sites surveyed, the queried database, and the project name. ABCA = Ausable Bayfield Conservation Authority; DFO = Fisheries and Oceans Canada; GLIER = Great Lakes Institute for Environmental Research; MU = McGill University; OMNR = Ontario Ministry of Natural Resources; ROM = Royal Ontario Museum; SLCC = Sea Lamprey Control Centre; TU = Trent University; UW = University of Waterloo; UWO = University of Western Ontario. *n* is the number of samples.*

<b>Population</b>	<b>Year</b>	<b><i>n</i></b>	<b>Organization</b>	<b>Project</b>
Ausable River	1928	1	ROM	Eastern Sand Darter record
Ausable River	2002	14	DFO	Ausable River Sampling 2002
Ausable River	2004	25	DFO	Ausable River Sampling 2004
Ausable River	2004	3	ABCA	Ausable Bayfield Conservation Authority
Ausable River	2004	2	UWO	Katie Stammeler Research
Ausable River	2005	3	DFO	Greenside Darter Sampling 2005
Ausable River	2007	13	DFO	Ausable River 2007
Ausable River	2007	11	DFO	Road Crossing 2007
Ausable River	2007	24	TU	Alan Dextrase Research
Ausable River	2008	5	ABCA	Ausable Bayfield Conservation Authority
Ausable River	2009	9	ABCA	Ausable Bayfield Conservation Authority
Ausable River	2012	3	DFO	2012 Fish/Mussel Overlap Study of Ausable River
Ausable River	2013	1	DFO	2013 Asian Carps Great Lakes Monitoring of Ausable River
Ausable River	2013	6	ABCA	Ausable Bayfield Conservation Authority
Ausable River	2014	2	DFO	2014 Asian Carps Great Lakes Monitoring of Ausable River
Ausable River	2014	3	ABCA	Ausable Bayfield Conservation Authority
Ausable River	2015	1	ABCA	Ausable Bayfield Conservation Authority
Ausable River	2015	4	DFO	2015 Asian Carps Great Lakes Monitoring of Ausable River
Ausable River	2016	5	DFO	2016 Asian Carps Great Lakes Monitoring of Ausable River
Ausable River	2017	1	DFO	2017 Asian Carps Great Lakes Monitoring of Ausable River
Ausable River	2017	36	DFO	2017 Eastern Sand Darter Ausable River Trawling
Ausable River	2018	1	DFO	2018 Asian Carps Great Lakes Monitoring of Ausable River
Ausable River	2018	9	DFO	2018 Eastern Sand Darter – Ausable River
Ausable River	2019	1	DFO	2019 Asian Carps Great Lakes Monitoring of Ausable River

<b>Population</b>	<b>Year</b>	<b>n</b>	<b>Organization</b>	<b>Project</b>
Ausable River	2019	3	ABCA	Ausable Bayfield Conservation Authority
Ausable River	2020	8	ABCA	Ausable Bayfield Conservation Authority
Ausable River	2022	3	ABCA	Ausable Bayfield Conservation Authority
Ausable River	2022	1	DFO	2022 Asian Carps Great Lakes Monitoring of Ausable River
Big Otter Creek	1923	1	ROM	Eastern Sand Darter record
Big Otter Creek	1955	2	ROM	Eastern Sand Darter records
Big Otter Creek	2008	14	TU	Alan Dextrase Research
Big Otter Creek	2018	43	DFO	2018 Eastern Sand Darter - Big Otter Creek
Big Otter Creek	2020	12	DFO	2020 Eastern Sand Darter Critical Habitat – Big Otter Creek
Big Otter Creek	2021	3	DFO	2021 Eastern Sand Darter – Big Otter Creek (Round Goby collections)
Catfish Creek	1922	1	ROM	Eastern Sand Darter record
Catfish Creek	1941	1	ROM	Eastern Sand Darter record
Catfish Creek	2002	7	DFO	Black Redhorse Status 2002
Catfish Creek	2008	4	SLCC	Sea Lamprey Control Centre
Catfish Creek	2016	2	TU	Alan Dextrase Research
Grand River	2002	2	DFO	Black Redhorse Status 2002
Grand River	2002	8	OMNR	River Redhorse 2002–2004
Grand River	2003	14	DFO	Eastern Sand Darter Grand River 2003
Grand River	2003	4	OMNR	River Redhorse 2002–2004
Grand River	2004	10	DFO	Grand River 2004
Grand River	2004	5	OMNR	River Redhorse 2002–2004
Grand River	2005	1	DFO	Greenside Darter Sampling 2005
Grand River	2007	151	TU	Alan Dextrase Research
Grand River	2007	15	DFO	Road Crossing 2007
Grand River	2008	39	DFO	Grand River Round Goby Project 2008
Grand River	2009	50	DFO	Grand River Trawling 2009
Grand River	2010	36	DFO	Grand River Trawling 2010
Grand River	2010	29	GLIER	SECT 73 SARA CA 10-005 and 10-005b
Grand River	2011	33	DFO	Grand River Trawling 2011
Grand River	2011	10	DFO	Silver Shiner Status Update 2011
Grand River	2013	49	DFO	2013 Eastern Sand Darter eDNA Survey of Grand River
Grand River	2013	32	DFO	2013 Round Goby Distribution Survey
Grand River	2014	37	DFO	2014 Round Goby Distribution Survey
Grand River	2014	14	OMNR	DFO-CA-14-00020

<b>Population</b>	<b>Year</b>	<b>n</b>	<b>Organization</b>	<b>Project</b>
Grand River	2015	2	OMNR	15-PCAA-00021
Grand River	2015	2	DFO	2015 Biodiversity Science Orientation
Grand River	2016	2	MU	16-PCAA-00009
Grand River	2016	1	OMNR	16-PCAA-00024
Grand River	2016	5	DFO	2016 Grand River eDNA
Grand River	2016	16	DFO	2016 Round Goby Distribution Survey
Grand River	2017	1	DFO	2017 Silver Shiner Status Update
Grand River	2018	6	DFO	2018 Mussel/Fish Overlap Study - Grand River
Grand River	2019	27	DFO	2019 Eastern Sand Darter Physiological Tolerances
Grand River	2021	2	DFO	2021 SAR Team Filming - DFO Comms
Grand River	2022	101	DFO	2022 Eastern Sand Darter Abundance - Grand River
Thames River	1983	20	ROM	Royal Ontario Museum 1983
Thames River	2003	54	DFO	Thames River Sampling 2003
Thames River	2004	142	DFO	Thames River 2004
Thames River	2005	90	UW	Mary Finch Research
Thames River	2005	336	DFO	Thames River (ESD)
Thames River	2005	13	DFO	Thames River First Nations - 2005
Thames River	2006	125	TU	Alan Dextrase Research
Thames River	2006	116	UW	Mary Finch Research
Thames River	2006	24	DFO	Thames River (ESD)
Thames River	2006	1	DFO	Thames River 2006
Thames River	2007	108	UW	Mary Finch Research
Thames River	2008	8	TU	Alan Dextrase Research
Thames River	2008	192	DFO	Northern Madtom Trapping 2008
Thames River	2008	42	DFO	Thames River Northern Madtom 2008
Thames River	2009	4	DFO	Eastern Sand Darter Population Genetics 2009
Thames River	2009	31	DFO	Thames River Trawling 2009
Thames River	2010	3	DFO	Mussel Trawl 2010
Thames River	2010	14	GLIER	SECT 73 SARA CA 10-005 and 10-005b
Thames River	2011	10	GLIER	SECT 73 SARA CA 11-015
Thames River	2011	5	OMNR	SECT 73 SARA CA 11-026
Thames River	2011	15	DFO	Silver Shiner Status Update – 2011
Thames River	2012	16	DFO	2012 Sea Lamprey Control
Thames River	2013	48	DFO	2013 Northern Madtom Population Genetics Survey
Thames River	2013	3	DFO	2013_SLCC

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<b>Population</b>	<b>Year</b>	<b><i>n</i></b>	<b>Organization</b>	<b>Project</b>
Thames River	2014	26	DFO	2014 Gravel Chub Critical Habitat
Thames River	2015	1	GLIER	15-PCAA-00024
Thames River	2015	1	DFO	15-PCAA-00007
Thames River	2015	3	DFO	2015 Mussel Fish Community Assessment
Thames River	2015	39	DFO	2015 Round Goby Distribution Survey
Thames River	2016	3	DFO	2016 Round Goby Distribution Survey
Thames River	2016	6	DFO	2016 Thames River Fish Community
Thames River	2017	6	DFO	2017 Mussel/Fish Community Assessment
Thames River	2018	43	OMNR	18-PCAA-00038
Thames River	2020	8	DFO	2020 Eastern Sand Darter Physiological Tolerances
Thames River	2021	4	UWO	21-PCAA-00028
Thames River	2022	5	DFO	2022 Asian Carps Great Lakes Monitoring of Thames River
Thames River	2022	20	DFO	2022 Northern Madtom - Thames River

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Table A2. Benthic macroinvertebrates (counts) captured from three sites in the Ausable River collected in the month of October. Counts are summed across all October samples. Samples collected in 2017-2020 (MAEXE1), 2017-2021 (MAGLAS1), and 2017 and 2019 (MAROCK1). Sampling conducted by the Ausable Bayfield Conservation Authority.

Phylum	Class	Order	Family	Genus	MAEXE1	MAGLAS1	MAROCK1
Annelida	Hirudinea	Rhynchobdellida	Glossiphoniidae	<i>Helobdella</i>	1	-	-
Annelida	Oligochaeta	Tubificida	Naididae	<i>Branchiura</i>	1	-	-
Annelida	Oligochaeta	Tubificida	Naididae	<i>Potamothrix</i>	6	-	-
Annelida	Oligochaeta	Tubificida	Naididae	-	1	10	-
Annelida	Oligochaeta	Tubificida	-	-	127	11	1
Annelida	Oligochaeta	-	-	-	1	1	-
Arthropoda	Acari	Trombidiformes	Lebertiidae	-	3	9	9
Arthropoda	Acari	Trombidiformes	Sperchontidae	-	-	1	-
Arthropoda	Acari	Trombidiformes	-	-	22	1	-
Arthropoda	Acari	-	-	-	11	9	7
Arthropoda	Insecta	Coleoptera	Elmidae	<i>Dubiraphia</i>	8	16	-
Arthropoda	Insecta	Coleoptera	Elmidae	<i>Optioservus</i>	1	-	-
Arthropoda	Insecta	Coleoptera	Elmidae	<i>Stenelmis</i>	16	14	3
Arthropoda	Insecta	Coleoptera	Elmidae	-	33	44	7
Arthropoda	Insecta	Coleoptera	Hydroptilidae	-	4	-	16
Arthropoda	Insecta	Coleoptera	Limnichidae	-	-	-	2
Arthropoda	Insecta	Coleoptera	Psephenidae	-	11	6	-
Arthropoda	Insecta	Coleoptera	-	-	31	17	-
Arthropoda	Insecta	Diptera	Athericidae	-	-	3	-
Arthropoda	Insecta	Diptera	Chironomidae	<i>Cricotopus</i>	24	2	-
Arthropoda	Insecta	Diptera	Chironomidae	-	388	83	54
Arthropoda	Insecta	Diptera	Simuliidae	-	7	-	1
Arthropoda	Insecta	Diptera	Tipulidae	-	1	-	10
Arthropoda	Insecta	Diptera	-	-	329	50	8
Arthropoda	Insecta	Ephemeroptera	Baetidae	<i>Acerpenna</i>	3	8	-
Arthropoda	Insecta	Ephemeroptera	Baetidae	<i>Baetis</i>	-	3	-
Arthropoda	Insecta	Ephemeroptera	Baetidae	-	6	5	9
Arthropoda	Insecta	Ephemeroptera	Caenidae	-	10	27	5
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	-	2	-	-

Phylum	Class	Order	Family	Genus	MAEXE1	MAGLAS1	MAROCK1
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	<i>Stenacron</i>	9	2	3
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	<i>Stenonema</i>	3	58	21
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	-	15	14	16
Arthropoda	Insecta	Ephemeroptera	Isonychiidae	<i>Isonychia</i>	-	-	6
Arthropoda	Insecta	Ephemeroptera	Isonychiidae	-	-	5	23
Arthropoda	Insecta	Ephemeroptera	Leptohyphidae	-	8	79	149
Arthropoda	Insecta	Ephemeroptera	-	-	52	86	-
Arthropoda	Insecta	Lepidoptera	Crambidae	-	5	-	4
Arthropoda	Insecta	Odonata	Calopterygidae	-	5	1	-
Arthropoda	Insecta	Odonata	Coenagrionidae	-	14	5	3
Arthropoda	Insecta	Odonata	-	-	13	1	-
Arthropoda	Insecta	Plecoptera	Capniidae	-	11	-	-
Arthropoda	Insecta	Plecoptera	Taeniopterygidae	-	-	39	2
Arthropoda	Insecta	Plecoptera	-	-	-	4	-
Arthropoda	Insecta	Trichoptera	Glossosomatidae	-	-	1	1
Arthropoda	Insecta	Trichoptera	Helicopsychidae	<i>Helicopsyche</i>	5	-	-
Arthropoda	Insecta	Trichoptera	Helicopsychidae	-	15	-	5
Arthropoda	Insecta	Trichoptera	Hydropsychidae	<i>Ceratopsyche</i>	-	3	23
Arthropoda	Insecta	Trichoptera	Hydropsychidae	<i>Cheumatopsyche</i>	28	-	-
Arthropoda	Insecta	Trichoptera	Hydropsychidae	<i>Hydropsyche</i>	1	-	-
Arthropoda	Insecta	Trichoptera	Hydropsychidae	-	64	60	21
Arthropoda	Insecta	Trichoptera	Hydroptilidae	-	-	256	-
Arthropoda	Insecta	Trichoptera	Leptoceridae	-	30	8	1
Arthropoda	Insecta	Trichoptera	Odontoceridae	-	4	-	-
Arthropoda	Insecta	Trichoptera	Philopotamidae	-	4	1	-
Arthropoda	Insecta	Trichoptera	Polycentropodidae	-	-	4	-
Arthropoda	Insecta	Trichoptera	-	-	36	15	-
Arthropoda	Malacostraca	Amphipoda	Crangonyctidae	-	2	-	-
Arthropoda	Malacostraca	Amphipoda	Hyalellidae	<i>Hyalella</i>	-	10	-
Arthropoda	Malacostraca	Amphipoda	Hyalellidae	-	-	1	-
Arthropoda	Malacostraca	Amphipoda	-	-	7	2	-
Arthropoda	Malacostraca	Decapoda	-	-	-	1	-

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Phylum	Class	Order	Family	Genus	MAEXE1	MAGLAS1	MAROCK1
Arthropoda	Malacostraca	Isopoda	Asellidae	-	2	-	-
Arthropoda	Malacostraca	Isopoda	-	-	2	-	-
Mollusca	Bivalvia	Sphaeriida	-	-	3	-	-
Mollusca	Bivalvia	Veneroida	Pisidiidae	<i>Musculium</i>	7	-	-
Mollusca	Bivalvia	Veneroida	Pisidiidae	<i>Sphaerium</i>	-	10	-
Mollusca	Bivalvia	Veneroida	Pisidiidae	-	6	13	6
Mollusca	Bivalvia	Veneroida	-	-	10	5	-
Mollusca	Gastropoda	Bosommatophora	Ancylidae	<i>Ferrissia</i>	1	-	-
Mollusca	Gastropoda	Bosommatophora	-	-	5	-	-
Platyhelminthes	Trepaxonemata	Tricladida	Dugesiidae	<i>Cura</i>	16	-	-
Platyhelminthes	Trepaxonemata	Tricladida	Dugesiidae	-	16	1	-
Platyhelminthes	Trepaxonemata	Tricladida	Planariidae	-	53	1	-
Platyhelminthes	Trepaxonemata	Tricladida	-	-	9	-	-

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Table A3. Benthic macroinvertebrates captured from six sites in Big Otter Creek in October 2022. Included are the total number captured (Count) and the number of sites where the macroinvertebrate was detected. Sampling conducted by the University of Toronto Scarborough.

Phylum	Class	Order	Family	Genus	Species	Count	No. of sites
Arthropoda	Insecta	Coleoptera	Elmidae	<i>Dubiraphia</i>	<i>parva</i>	2	2
Arthropoda	Insecta	Coleoptera	Elmidae	<i>Macronychus</i>	<i>glabratus</i>	2	2
Arthropoda	Insecta	Coleoptera	Elmidae	<i>Stenelmis</i>	sp.	1	1
Arthropoda	Insecta	Coleoptera	Empididae	<i>Optioservus</i>	sp.	5	1
Arthropoda	Insecta	Diptera	Athericidae	<i>Antherix</i>	<i>lantha</i>	1	1
Arthropoda	Insecta	Diptera	Chironomidae	<i>Ablabesmyia</i>	sp.	2	1
Arthropoda	Insecta	Diptera	Chironomidae	<i>Bezzia</i>	sp.	1	1
Arthropoda	Insecta	Diptera	Chironomidae	<i>Chironomus</i>	sp.	33	5
Arthropoda	Insecta	Diptera	Chironomidae	<i>Diamesa</i>	sp.	3	2
Arthropoda	Insecta	Diptera	Empididae	<i>Hemerodromia</i>	sp.	1	1
Arthropoda	Insecta	Diptera	Limoniidae	<i>Antocha</i>	<i>saxicola</i>	1	1
Arthropoda	Insecta	Diptera	Tabinidae	<i>Chrysops</i>	<i>exitans</i>	1	1
Arthropoda	Insecta	Diptera	Tabinidae	<i>Tabanus</i>	<i>septantrionalis</i>	4	2
Arthropoda	Insecta	Diptera	Tipulidea	<i>Tipula</i>	<i>abdominalis</i>	13	3
Arthropoda	Insecta	Ephemeroptera	Ameletidae	<i>Ameletus</i>	sp.	3	3
Arthropoda	Insecta	Ephemeroptera	Caenidae	<i>Caenis</i>	<i>horaria</i>	1	1
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	<i>Heptagenia</i>	<i>elegantula</i>	2	1
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	<i>Heptagenia</i>	<i>flarenseens</i>	6	1
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	<i>Heptagenia</i>	sp.	1	1
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	<i>Leucrocuta</i>	sp.	7	2
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	<i>Stenacron</i>	<i>pallidum</i>	2	1
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	<i>Stenacron</i>	sp.	1	1
Arthropoda	Insecta	Hemiptera	Corixidae	<i>Hesperocorixa</i>	<i>vulgaris</i>	323	4
Arthropoda	Insecta	Megaloptera	Sialidae	<i>Sialis</i>	sp.	5	1
Arthropoda	Insecta	Odonata	Calopterygidae	<i>Calopteryx</i>	<i>virgo</i>	2	2
Arthropoda	Insecta	Trichoptera	Hydropsychidae	<i>Chematopsyche</i>	<i>analis</i>	42	3
Arthropoda	Insecta	Trichoptera	Hydropsychidae	<i>Chematopsyche</i>	<i>hageni</i>	4	1
Arthropoda	Insecta	Trichoptera	Hydropsychidae	<i>Chematopsyche</i>	<i>leonardi</i>	3	1
Arthropoda	Insecta	Trichoptera	Hydropsychidae	<i>Hydropsyche</i>	<i>alheda</i>	4	1
Arthropoda	Insecta	Trichoptera	Hydropsychidae	<i>Hydropsyche</i>	<i>alternans</i>	9	1
Arthropoda	Insecta	Trichoptera	Hydropsychidae	<i>Hydropsyche</i>	<i>betteni</i>	1	1

Phylum	Class	Order	Family	Genus	Species	Count	No. of sites
Arthropoda	Insecta	Trichoptera	Hydropsychidae	<i>Hydropsyche</i>	<i>bronta</i>	7	3
Arthropoda	Insecta	Trichoptera	Hydropsychidae	<i>Hydropsyche</i>	<i>carolina</i>	40	1
Arthropoda	Insecta	Trichoptera	Hydropsychidae	<i>Hydropsyche</i>	<i>dicanthra</i>	6	1
Arthropoda	Insecta	Trichoptera	Hydropsychidae	<i>Hydropsyche</i>	<i>hageni</i>	10	1
Arthropoda	Insecta	Trichoptera	Hydropsychidae	<i>Hydropsyche</i>	<i>leonardi</i>	12	2
Arthropoda	Insecta	Trichoptera	Hydropsychidae	<i>Symphitopsyche</i>	<i>bifida</i>	15	1
Arthropoda	Insecta	Trichoptera	Rhyacophilidae	<i>Rhyacophila</i>	<i>meyeri</i>	1	1
Arthropoda	Malacostraca	Amphipoda	Gammaridae	<i>Gammarus</i>	<i>fossarum</i>	1	1
Arthropoda	Malacostraca	Amphipoda	Gammaridae	<i>Gammarus</i>	<i>pulex</i>	8	2
Arthropoda	Malacostraca	Decapoda	Cambaridae	<i>Cambarus</i>	<i>robustus</i>	1	1
Mollusca	Bivalvia	Sphaeriida	Sphaeriidae	<i>Psidium</i>	sp.	2	1
Mollusca	Bivalvia	Sphaeriida	Sphaeriidae	<i>Sphaerium</i>	<i>corneum</i>	1	1
Mollusca	Bivalvia	Sphaeriida	Sphaeriidae	<i>Sphaerium</i>	<i>occidentale</i>	5	2
Mollusca	Gastropoda	Basommatophora	Planorbidae	<i>Anisus</i>	sp.	5	1
Mollusca	Gastropoda	Heterostropha	Valvatidae	<i>Valvata</i>	<i>tricarinata</i>	1	1

Table A4. Checklist of species detected in the Grand River, Thames River, Ausable River, Big Otter Creek, and Catfish Creek. Data included in this checklist include the biodiversity database ([Fish Biodiversity Database](#)), graduate student database, and external database held by DFO's Great Lakes Laboratory for Fisheries and Aquatic Sciences. Included are scientific name, common name, and abbreviations. ✓ = species was previously detected within the river; Ø = species not recorded in the queried databases. Note that fish occurrences in the Grand and Thames rivers was limited to areas of Eastern Sand Darter critical habitat. Fish occurrences in the Ausable River were only considered from surveys upstream of 'The Cut' to Exeter dam. Fish occurrences in Big Otter Creek were between Vienna and Otterville. T = true and F = False.

Scientific name	Common name	Predator?	Competitor?	Grand River	Thames River	Ausable River	Big Otter Creek	Catfish Creek
<i>Ambloplites rupestris</i>	Rock Bass	T	T	Ø	✓	✓	✓	✓
<i>Ameiurus melas</i>	Black Bullhead	F	T	✓	✓	✓	Ø	Ø
<i>Ameiurus natalis</i>	Yellow Bullhead	F	T	Ø	✓	✓	Ø	Ø
<i>Ameiurus nebulosus</i>	Brown Bullhead	F	T	✓	✓	✓	Ø	✓
<i>Ammocrypta pellucida</i>	Eastern Sand Darter	F	F	✓	✓	Ø	Ø	Ø
<i>Aplodinotus grunniens</i>	Freshwater Drum	F	F	✓	✓	✓	Ø	✓
<i>Campostoma anomalum</i>	Central Stoneroller	F	F	✓	✓	✓	Ø	Ø
<i>Carassius auratus</i>	Goldfish	F	T	Ø	✓	Ø	Ø	Ø
<i>Carpodes cyprinus</i>	Quillback	F	T	Ø	✓	✓	Ø	✓
<i>Catostomus catostomus</i>	Longnose Sucker	F	T	✓	✓	Ø	Ø	Ø
<i>Catostomus commersonii</i>	White Sucker	F	T	✓	✓	✓	✓	✓
<i>Chrosomus eos</i>	Northern Redbelly Dace	F	T	Ø	Ø	✓	Ø	Ø
<i>Culaea inconstans</i>	Brook Stickleback	F	T	✓	✓	✓	Ø	Ø
<i>Cyprinella spiloptera</i>	Spotfin Shiner	F	F	✓	✓	✓	✓	✓
<i>Cyprinus carpio</i>	Common Carp	F	T	✓	✓	✓	Ø	✓
<i>Dorosoma cepedianum</i>	Gizzard Shad	F	F	✓	✓	✓	Ø	✓
<i>Esox americanus vermiculatus</i>	Grass Pickerel	T	T	Ø	Ø	✓	Ø	Ø
<i>Esox lucius</i>	Northern Pike	T	F	✓	✓	✓	Ø	Ø
<i>Etheostoma blennioides</i>	Greenside Darter	F	F	✓	✓	✓	Ø	Ø
<i>Etheostoma caeruleum</i>	Rainbow Darter	F	T	✓	✓	✓	Ø	Ø
<i>Etheostoma exile</i>	Iowa Darter	F	T	Ø	Ø	✓	Ø	Ø
<i>Etheostoma flabellare</i>	Fantail Darter	F	F	✓	✓	Ø	Ø	Ø
<i>Etheostoma nigrum</i>	Johnny Darter	F	T	✓	✓	✓	✓	✓
<i>Etheostoma olmstedii</i>	Tessellated Darter	F	T	Ø	✓	Ø	Ø	Ø
<i>Hiodon alosoides</i>	Goldeye	T	F	Ø	✓	Ø	Ø	Ø
<i>Hiodon tergisus</i>	Mooneye	T	F	✓	✓	Ø	Ø	Ø

Scientific name	Common name	Predator?	Competitor?	Grand River	Thames River	Ausable River	Big Otter Creek	Catfish Creek
<i>Hypentelium nigricans</i>	Northern Hogsucker	F	T	✓	✓	✓	✓	✓
<i>Ictalurus punctatus</i>	Channel Catfish	T	T	✓	✓	✓	∅	∅
<i>Labidesthes sicculus</i>	Brook Silverside	F	F	✓	✓	✓	✓	∅
<i>Lepisosteus osseus</i>	Longnose Gar	T	F	✓	✓	∅	∅	✓
<i>Lepomis cyanellus</i>	Green Sunfish	T	T	✓	✓	✓	∅	✓
<i>Lepomis gibbosus</i>	Pumpkinseed	F	T	✓	✓	✓	✓	✓
<i>Lepomis macrochirus</i>	Bluegill	F	T	✓	✓	✓	✓	✓
<i>Lepomis megalotis</i>	Longear Sunfish	F	T	∅	∅	✓	∅	∅
<i>Lepomis peltastes</i>	Northern Sunfish	F	T	✓	✓	✓	∅	∅
<i>Lethenteron appendix</i>	American Brook Lamprey	F	F	✓	✓	✓	∅	∅
<i>Luxilus chrysocephalus</i>	Striped Shiner	F	T	✓	✓	✓	∅	∅
<i>Luxilus cornutus</i>	Common Shiner	F	T	✓	✓	✓	✓	✓
<i>Lythrurus umbratilis</i>	Redfin Shiner	F	F	∅	✓	✓	∅	∅
<i>Micropterus dolomieu</i>	Smallmouth Bass	T	F	✓	✓	✓	✓	✓
<i>Micropterus salmoides</i>	Largemouth Bass	T	F	✓	✓	✓	∅	✓
<i>Minytrema melanops</i>	Spotted Sucker	F	T	∅	✓	∅	∅	∅
<i>Morone americana</i>	White Perch	T	T	∅	✓	✓	✓	∅
<i>Morone chrysops</i>	White Bass	T	F	∅	✓	✓	∅	∅
<i>Moxostoma anisurum</i>	Silver Redhorse	F	T	✓	✓	✓	∅	✓
<i>Moxostoma carinatum</i>	River Redhorse	F	T	✓	✓	∅	∅	∅
<i>Moxostoma duquesnei</i>	Black Redhorse	F	T	✓	✓	✓	∅	∅
<i>Moxostoma erythrurum</i>	Golden Redhorse	F	T	✓	✓	✓	∅	✓
<i>Moxostoma macrolepidotum</i>	Shorthead Redhorse	F	T	✓	✓	✓	✓	✓
<i>Moxostoma valenciennesi</i>	Greater Redhorse	F	T	✓	✓	✓	✓	∅
<i>Neogobius melanostomus</i>	Round Goby	F	T	✓	✓	✓	✓	∅
<i>Nocomis biguttatus</i>	Hornyhead Chub	F	T	✓	✓	✓	∅	∅
<i>Nocomis micropogon</i>	River Chub	F	T	✓	✓	✓	✓	∅
<i>Notemigonus crysoleucas</i>	Golden Shiner	F	F	✓	✓	∅	✓	✓
<i>Notropis atherinoides</i>	Emerald Shiner	F	F	✓	✓	✓	✓	∅
<i>Notropis buchanani</i>	Ghost Shiner	F	F	✓	✓	✓	∅	∅
<i>Notropis heterodon</i>	Blackchin Shiner	F	F	∅	∅	✓	∅	∅

Scientific name	Common name	Predator?	Competitor?	Grand River	Thames River	Ausable River	Big Otter Creek	Catfish Creek
<i>Notropis heterolepis</i>	Blacknose Shiner	F	F	∅	∅	✓	∅	∅
<i>Notropis hudsonius</i>	Spottail Shiner	F	F	✓	✓	∅	✓	∅
<i>Notropis photogenis</i>	Silver Shiner	F	F	✓	✓	∅	∅	∅
<i>Notropis rubellus</i>	Rosyface Shiner	F	F	✓	✓	✓	✓	∅
<i>Notropis stramineus</i>	Sand Shiner	F	F	∅	∅	∅	✓	∅
<i>Notropis volucellus</i>	Mimic Shiner	F	F	✓	✓	✓	✓	∅
<i>Noturus flavus</i>	Stonecat	T	T	✓	✓	✓	✓	✓
<i>Noturus gyrinus</i>	Tadpole Madtom	F	T	✓	∅	✓	✓	∅
<i>Noturus miurus</i>	Brindled Madtom	F	T	✓	✓	∅	∅	∅
<i>Noturus stigmosus</i>	Northern Madtom	F	T	∅	✓	∅	∅	∅
<i>Oncorhynchus mykiss</i>	Rainbow Trout	T	T	∅	✓	✓	✓	∅
<i>Oncorhynchus tshawytscha</i>	Chinook Salmon	T	F	∅	∅	✓	∅	∅
<i>Perca flavescens</i>	Yellow Perch	T	T	✓	✓	✓	✓	✓
<i>Percina caprodes</i>	Logperch	F	T	✓	✓	✓	✓	✓
<i>Percina maculata</i>	Blackside Darter	F	T	✓	✓	✓	✓	✓
<i>Percina shumardi</i>	River Darter	F	F	∅	✓	∅	∅	∅
<i>Percopsis omiscomaycus</i>	Trout-perch	F	T	✓	✓	✓	✓	✓
<i>Petromyzon marinus</i>	Sea Lamprey	F	F	∅	✓	∅	∅	✓
<i>Pimephales notatus</i>	Bluntnose Minnow	F	T	✓	✓	✓	✓	✓
<i>Pimephales promelas</i>	Fathead Minnow	F	T	✓	✓	✓	✓	∅
<i>Pomoxis annularis</i>	White Crappie	T	F	✓	✓	✓	∅	∅
<i>Pomoxis nigromaculatus</i>	Black Crappie	T	F	✓	✓	✓	∅	✓
<i>Rhinichthys atratulus</i>	Eastern Blacknose Dace	F	T	✓	✓	✓	✓	∅
<i>Rhinichthys cataractae</i>	Longnose Dace	F	F	✓	✓	✓	✓	∅
<i>Salmo trutta</i>	Brown Trout	T	F	∅	∅	✓	∅	∅
<i>Sander vitreus</i>	Walleye	T	F	✓	✓	✓	∅	∅
<i>Semotilus atromaculatus</i>	Creek Chub	F	T	✓	✓	✓	✓	✓

## APPENDIX II – SUMMARY OF FISH SAMPLING EFFORT IN CATFISH CREEK

Backpack and boat electrofishing methods were used in July 2002 to sample Black Redhorse between the river mouth and downstream of the Springwater Pond dam. A total of 107 fishes encompassing 28 species were captured across the eight sites surveyed. Gizzard Shad (*Dorosoma cepedianum*) was captured the most ( $n = 23$ ), with fewer than 10 individuals captured for the remaining 27 species (Table A4 **Error! Reference source not found.**); Eastern Sand Darter was not detected. Three repeat seine hauls were performed at two sites in Catfish Creek near the Pleasant Valley Road Crossing in Central Elgin, Ontario in July 2008 (Table A1). Two Spottfin Shiner (*Cyprinella spiloptera*) and one Blackside Darter (*Percina maculata*) were captured across the six hauls. Finally, six fish species were captured in April 2016 across four surveys: Brown Bullhead (*Ameiurus nebulosus*;  $n = 2$  individuals), White Sucker (*Catostomus commersonii*;  $n = 26$ ), Pumpkinseed (*Lepomis gibbosus*;  $n = 1$ ), Stonecat (*Noturus flavus*;  $n = 692$ ), Trout-perch (*Percopsis omiscomaycus*;  $n = 2$ ), and Sea Lamprey (*Petromyzon marinus*;  $n = 21$ ; Figure A1). Habitat and water quality measurements from Catfish Creek are generally unavailable; however, the system is known to be highly turbid.

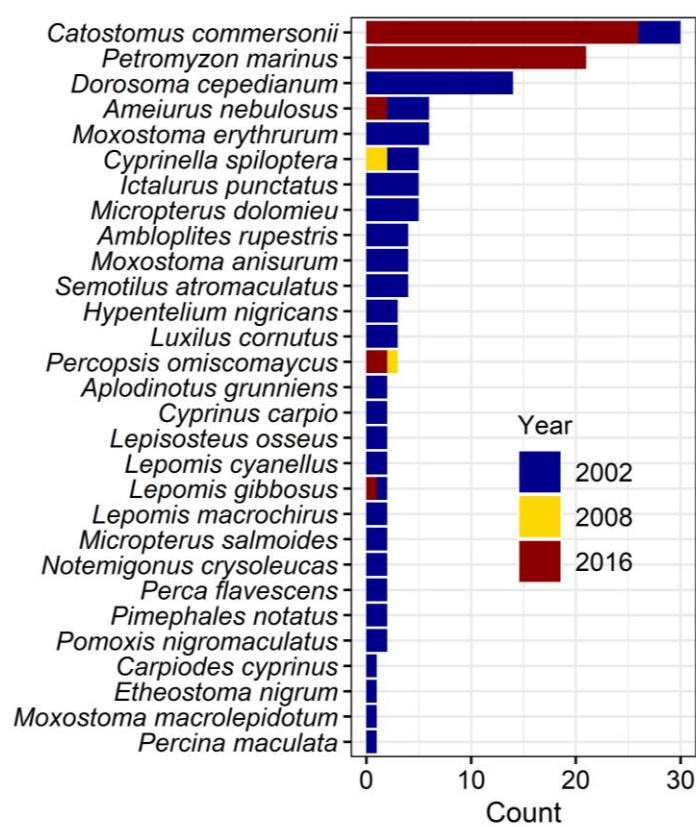


Figure A1. Fish capture data from Catfish Creek for 2002 (dark blue; 8 sites), 2008 (gold; 2 sites), and 2016 (dark red; 4 sites). *Noturus flavus* was excluded from this plot (2002: 2 individuals; 2016: 692 individuals).

## APPENDIX III – OVERVIEW OF MODIFIED DELPHI APPROACH

### INTRODUCTION TO THE MODIFIED MINI-DELPHI APPROACH

Scientific knowledge about how to improve the survival and recovery of species listed under the *Species at Risk Act* (SARA) is often incomplete, but management decisions must be made despite numerous uncertainties. Management decisions under high uncertainty can be improved by incorporating a structured consensus method with a group of experts or specialists. Broadly, consensus methods are defined as structured qualitative approaches to characterize the agreement among experts on complex issues, and in doing so, derive quantitative information (Jones and Hunter 1995). Although the application of consensus methods to conservation problems are relatively rare (Mukherjee et al. 2015), the approaches provide several benefits when addressing difficult questions related to species conservation and management.

The Delphi method is a type of consensus method commonly used to structure groups of individuals to collectively answer complex problems (Hasson et al. 2000, Mukherjee et al. 2015; Figure A2). The method was popularized ~ 50 years ago with the objective of improving technological forecasting (Dalkey and Helmer 1963, Linstone and Turoff 1975, Rowe and Wright 2011) and has now become a common approach across disciplines (e.g., health and medicine: Murphy et al. 2019, Lazarus et al. 2022). Broadly, the Delphi method uses questionnaires and controlled feedback sequentially to narrow down uncertainty in the answers to complex questions. The first stage in the Delphi method is preparation (Figure A2), where the question or problem is defined, and experts are chosen to participate based on their knowledge and experience. The number of participants can vary and is often limited by resources but is generally recommended to include seven or more experts (Powell 2003, Mukherjee et al. 2015). In addition to objective development and invitations for the participation of experts, the first step also requires development of the initial set of questions for the expert panel. Typically, the first set of questions are broad and can help shape the direction of subsequent questionnaires but can also be more structured depending on the scenario (Powell 2003).

#### Traditional Delphi Method

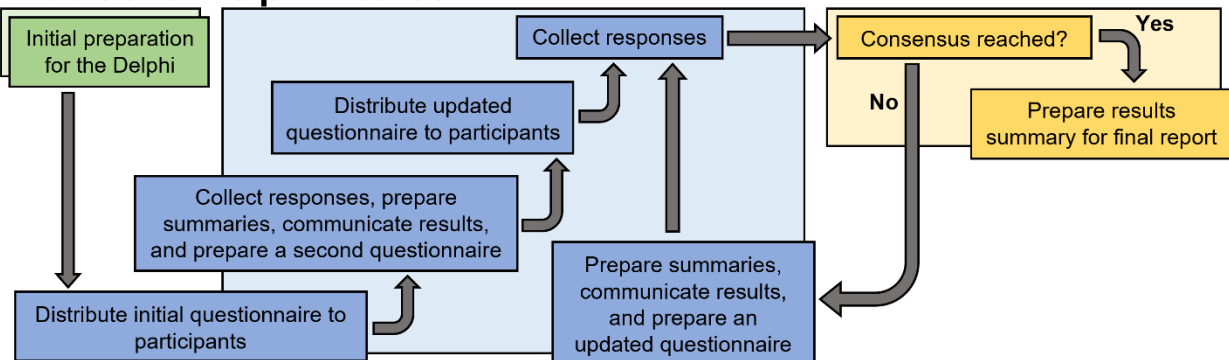


Figure A2. Flowchart of the traditional Delphi technique. Colours correspond with the flowchart in Figure A3. Initial preparation (green), application (blue), and completion (consensus; yellow).

Following completion, questionnaires are collected, and the responses are aggregated and summarized (Figure A2). Methods for analyzing the results differ based on the structure of the questions and the objectives of using the Delphi method. Ultimately, the objective of presenting the results to the participants is to provide the expert panel with sufficient information to allow each individual the ability to consider other expert perspectives and weigh those perspectives against their own scores. After presenting the results, the expert panel is allowed the

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opportunity to revise their answers in a second view of the questionnaire. After completion, results of the questionnaires are aggregated across the expert participants. If consensus was reached across experts, results are summarized and communicated in a final report. If, however, consensus was not reached, the expert panel is provided updated results and summaries, and allowed the opportunity to revise their answers in a third view of the questionnaire (Figure A2). This process is repeated until consensus is reached or can no longer be continued, and dissenting views are recorded.

In its conventional use, the Delphi method is anonymous and encompasses at least two rounds of structured questionnaires, each followed by aggregation of responses and anonymous feedback to the participants. Anonymity is maintained between experts by having participants complete the questionnaires independently, without knowledge of who is serving on the panel (i.e., no in-person meeting). The results of the questionnaires are summarized by the facilitators and provided to the participants, where the answers to each question across participants are provided (anonymously) that include summaries of participants' perspectives. The more information provided by the participants to support their perspective, the more informative the data summaries can be, which can ultimately speed up the process. Therefore, precision is needed when developing the questionnaire to ensure that the information generated aligns with the necessary data for informing decision-making.

## **MODIFIED MINI-DELPHI METHODS**

Many variants of the Delphi method have been developed to allow the examination of problems with unique contexts and (or) objectives (Rauch 1979, Mukherjee et al. 2015, Strasser 2017). The variant being adapted for use in the Canadian Science Advisory Secretariat (CSAS) peer-review meeting on Eastern Sand Darter (*Ammocrypta pellucida*) reintroduction is termed a 'mini-Delphi', or an 'Estimate-Talk-Estimate' (Gallego and Bueno 2014, Strasser 2017). A mini-Delphi consists of a physical [or virtual] meeting of experts to provide individual input with subsequent discussions or debates regarding the aggregated answers (Gallego and Bueno 2014, Strasser 2017). In this form, the actual responses to questions can be kept anonymous; however, given the opportunity and need for discussion, complete anonymity is lost.

A modified mini-Delphi method was used to inform decisions on the use of reintroduction for improving the survival or recovery of Eastern Sand Darter in southwestern Ontario (Figure A3). This process was divided into three stages:

1. Before meeting: Preparation
2. During meeting: Application
3. After meeting: Completion

## Modified Mini-Delphi Method

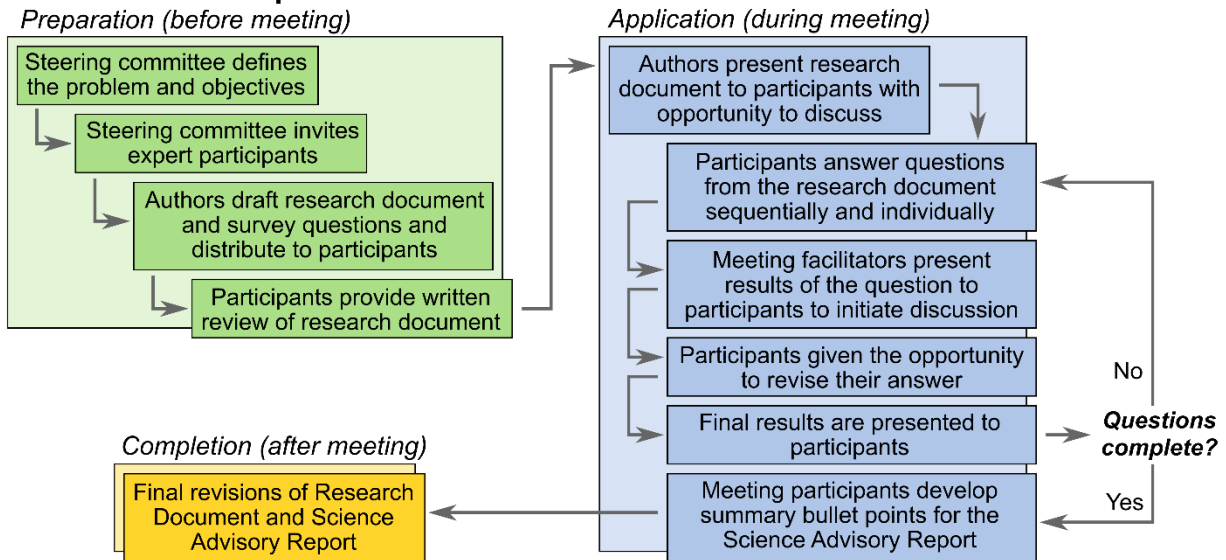


Figure A3. Modified mini-Delphi method being used to consider the benefits, feasibility, and risks of reintroduction for Eastern Sand Darter. Three stages are outlined for before (preparation; green), during (application; blue), and after (completion; yellow) the CSAS meeting has concluded.

The first step for using the modified mini-Delphi method was for the steering committee to identify and refine the objectives for the meeting (Figure A3), beginning at the request for peer-reviewed science information and/or advice, and outlined in the Terms of Reference for the CSAS meeting. With the research objectives identified, the steering committee developed a list of potential expert participants and a facilitator for leading the meeting and solicited their participation. Next, the authors drafted a working paper that provided background information relevant to the objectives and the questions in the survey. The working paper drew heavily from the previously developed guidance document for evaluating the ecological benefits and risks of conservation translocation for SARA-listed freshwater fishes and mussels (Lamothe et al. 2023). Prior to the meeting, participants provided written reviews of the working paper that identified any major considerations or suggested information that was absent. The author team prepared responses and revisions prior to the meeting as best as possible given time constraints.

During the first day of the CSAS meeting, the author team presented the contents of the working paper, including any suggested revisions, to facilitate discussion on the material. Following the overview of the research, participants were asked to independently answer the survey questions identified in the working paper. Questions asked to participants were worded to ensure that a measurement of participant uncertainty could be derived. For example, participants were asked:

**Question 1:** What is the probability that Eastern Sand Darter population abundance in the Grand River is greater than 25,000 age-1+ individuals?

In response to this question, participants allocated 100 points between five categories to indicate how likely the statement was true while simultaneously providing a level of certainty with that perceived judgement:

1. Very low probability (< 5%)
2. Low probability (5–33%)
3. Medium probability (34–66%)

4. High probability (67–95%)
5. Very high probability (> 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 hesitation or uncertainty in that answer, they may allocate their points more heavily toward category 4, high likelihood (67–95%; Table A5). Alternatively, if there was no relevant information about Question 1 and the expert was completely uncertain about the true probability, they allocated their points uniformly across all categories (Expert 2; Table A5). Approaching the questions and responses in this format provides answers to challenging questions from a panel with significant expertise and a measure of certainty that can be considered at the individual and group level to inform future management decisions.

*Table A3. Example responses to Question 1 from two experts. Each expert allocated 100 points to five probability categories. Question 1: What is the probability that Eastern Sand Darter population abundance in the Grand River is greater than 25,000 age-1+ individuals?*

<b>Probability</b>	<b>Expert 1 scores</b>	<b>Expert 2 scores</b>
Very low (< 5%)	0	20
Low (5% – 33%)	10	20
Medium (34% – 66%)	25	20
High (67% – 95%)	40	20
Very high (> 95%)	25	20
<b>SUM</b>	<b>100</b>	<b>100</b>

Feedback and summaries were presented for each question after it was answered. Simple histograms of the relative frequency of scores for each probability category summed across participants (e.g., Figure A4) were generated that informed participants on the group perspective with the goal of facilitating discussion on the probability and uncertainty assigned for each question. Once the group of participants felt that the follow-up discussion on the results for a particular question was sufficient, participants had the opportunity to revise their scores. Although contrasting with a conventional Delphi approach that requires consensus for each question, the goal of using the modified mini-Delphi approach for the Eastern Sand Darter reintroduction CSAS was to gain an informed picture of the probability and certainty around the ability to achieve a successful reintroduction and how that reintroduction may affect survival and recovery of the species.

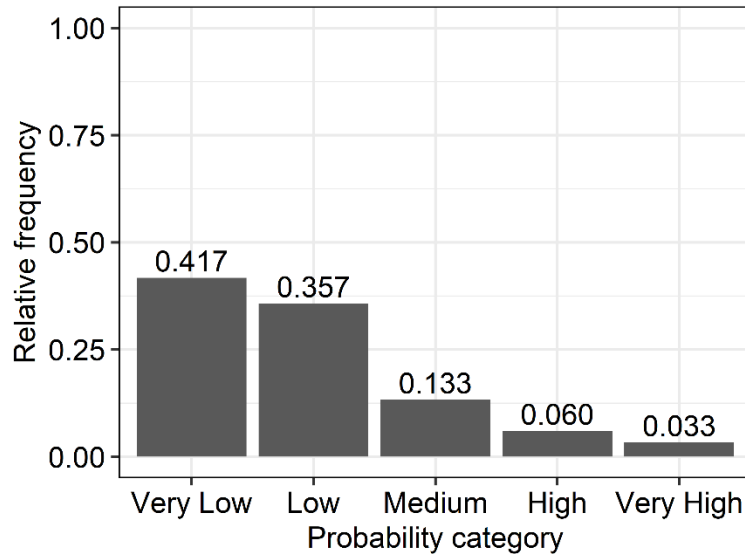


Figure A4. Example of a histogram displaying simulated probability estimates (x-axis) for Question 1 related to source population abundance.

The participants approved the results generated from the mini-Delphi method during the meeting, and the results are presented in the Science Advisory Report. The discussions that took place during the mini-Delphi process was captured by rapporteurs and published as a Proceedings document.

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