Proceedings of the Regional peer-review meeting for evaluating the ecological benefits and risks of an experimental Eastern Sand Darter (*Ammocrypta pellucida*) reintroduction in Ontario

Julia E. Colm, Karl A. Lamothe, Jason M. Barnucz, Amy Boyko, Jeremy E. Broome, Alan J. Dextrase, Jennifer Diment, Britney L. Firth, Paul Gagnon, William F. Glass, Paul Grant, Kari Jean, Marten A. Koops, Nicholas E. Mandrak, Todd J. Morris, Gilles Olivier, Trevor E. Pitcher, Lindsay Potts, Scott M. Reid, Shawn K. Staton, Wendylee Stott, Adam S. van der Lee, Chris C. Wilson, and D. Andrew R. Drake

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A Canadian Science Advisory Secretariat (CSAS) regional peer-review meeting was held on April 3–6th, 2023 via Microsoft Teams to evaluate the ecological benefits, feasibility, and risks of an experimental Eastern Sand Darter (*Ammocrypta pellucida*) reintroduction in Ontario. This CSAS was a follow-up implementation case study to a national CSAS peer-review meeting Decision Support Framework for the Conservation Translocation of SARA-listed Freshwater Fishes and Mussels. The objectives of the meeting were to evaluate the ecological factors that could influence the successful reintroduction of Eastern Sand Darter into candidate recipient locations from candidate source populations; evaluate the risks associated with such a reintroduction to both Eastern Sand Darter and the broader ecosystem at source and recipient locations; and, to evaluate the change in survival or recovery of Eastern Sand Darter resulting from re-establishment in a formerly occupied location. Building from the framework developed during the national Conservation Translocation CSAS, questions related to the probability of success and unintended ecological consequences were tailored to Eastern Sand Darter in two candidate source rivers and two candidate recipient rivers (where the species historically occurred). Relevant data and analyses to inform each question were compiled and presented in a working paper, and distributed to participants in advance of the peer-review meeting. Following a review of this information during the meeting, a quantitative, structured expert judgement (modified Delphi) exercise was undertaken to capture expert opinion and associated uncertainty on the ecological benefits and risk factors associated with an experimental reintroduction.

RÉSUMÉ

Colm, J.E., Lamothe, K.A., Barnucz, J.M., Boyko, A., Broome, J.E., Dextrase, A.J., Diment, J., Firth, B.L., Gagnon, P., Glass, W.F., Grant, P., Jean, K., Koops, M.A., Mandrak, N.E., Morris, T.J., Olivier, G., Pitcher, T.E., Potts, L., Reid, S.M., Staton S.K., Stott, W., van der Lee, A.S., Wilson, C.S., and Drake, D.A.R. 2025. Proceedings of the Regional peer-review meeting for evaluating the ecological benefits and risks of an experimental Eastern Sand Darter (Ammocrypta pellucida) reintroduction in Ontario. Can. Data Rep. Fish. Aquat. Sci. 3301: v + 56 p. https://doi.org/10.60825/7290-4m49

canadien des avis scientifiques (SCAS) a tenu du 3 au 6 avril 2023 par Microsoft Teams une réunion d'examen régional par les pairs pour évaluer les avantages écologiques, la faisabilité et les risques d'une réintroduction expérimentale du dard de sable (*Ammocrypta pellucida*) en Ontario. Ce processus du SCAS portait sur une étude de cas de mise en œuvre menée à la suite d'une réunion d'examen national par les pairs du SCAS sur le Cadre décisionnel pour la translocation aux fins de la conservation de poissons et de moules d'eau douce inscrits sur la liste de la LEP. Les objectifs de la réunion étaient d'évaluer les facteurs écologiques qui pourraient influer sur la réussite de la réintroduction du dard de sable aux emplacements récepteurs possibles à partir de populations sources candidates, d'évaluer les risques associés à une telle réintroduction à la fois pour le dard de sable et pour l'écosystème en général aux emplacements sources et récepteurs, et d'évaluer le changement dans la survie ou le rétablissement du dard de sable résultant de sa réintroduction dans un emplacement qu'il occupait autrefois. En reprenant le cadre élaboré au cours du processus national du SCAS sur le cadre décisionnel pour la translocation aux fins de la conservation, les questions relatives à la probabilité de réussite et aux conséquences écologiques imprévues ont été adaptées au dard de sable dans deux cours d'eau sources candidats et deux cours d'eau récepteurs candidats (où l'espèce était présente par le passé). Les données et les analyses pertinentes pour chaque question ont été compilées et présentées dans un document de travail et distribuées aux participants avant la réunion d'examen par les pairs. À la suite de l'examen de cette information pendant la réunion, un exercice quantitatif et structuré d'avis d'experts (approche Delphi modifiée) a été entrepris pour saisir l'avis d'experts et l'incertitude connexe sur les avantages écologiques et les facteurs de risque associés à une réintroduction expérimentale.

INTRODUCTION

Eastern Sand Darter (*Ammocrypta pellucida*) (Ontario populations) is listed as Threatened under Canada's *Species at Risk Act* (SARA). Reintroduction has been identified as a recovery measure in the Recovery Strategy for this species, pending an evaluation of feasibility. Fisheries and Oceans Canada (DFO)'s Species at Risk Program requested science advice on the feasibility of reintroducing Eastern Sand Darter into historically occupied locations. A Canadian Science Advisory Secretariat (CSAS) regional peer-review meeting was held on April 3–6, 2022 via Microsoft Teams. The objectives of this meeting were to evaluate the ecological benefits, feasibility, and risks of an experimental reintroduction of Eastern Sand Darter; further context and scope are described in the Terms of Reference (Appendix 1). A working paper that compiled relevant information pertaining to an Eastern Sand Darter reintroduction was distributed to participants for review, and then a modified mini-Delphi approach was used to gather expert opinion and capture uncertainties for 30 questions related to the probability of success and the probability of unintended consequences. The meeting generally followed the agenda in Appendix 2. Participants included staff from DFO Science, Species at Risk Program, and Fish and Fish Habitat Protection Program; Ontario Ministry of Natural Resources and Forestry (Aquatic Research and Monitoring Section); Ausable Bayfield Conservation Authority; Long Point Region Conservation Authority; and academia (Appendix 3).

This manuscript report summarizes the relevant discussions from the meeting, including from the modified mini-Delphi exercise, and presents recommended revisions to be made to the associated Research Document. The Research Document and Science Advisory Report resulting from this science advisory meeting are published on the DFO Canadian Science Advisory Secretariat (CSAS) website.

PRESENTATIONS

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

Presenter: Karl Lamothe

Abstract

Species Recovery Strategies frequently identify the need for reintroductions to improve the survival and recovery of species listed under the *Species at Risk Act*; however, there are only a few examples of reintroductions being implemented. Reintroduction is proposed as a potential recovery measure for Eastern Sand Darter because is has experienced several extirpations across its Canadian range. The lack of reintroduction efforts over the last two decades has been at least partially due to incomplete knowledge of the ecological requirements for the species and approaches for adequately assessing the ecological benefits, feasibility, and risks of performing the reintroduction. However, knowledge of Eastern Sand Darter ecology has improved substantially over the past 15 years, and the recent development of national guidance around when, and in which scenarios, reintroduction could be considered as a recovery tool provides an opportunity to evaluate the reintroduction. As such, the objective of the research document was to use the decision-support framework to evaluate the potential benefits, feasibility, and risks of an experimental Eastern Sand Darter reintroduction in Ontario. This includes assembling information on the ecological factors that could influence the success of the recovery action, the ecological risks for the species and broader ecosystem at source and recipient locations, and the change in survival or recovery of Eastern Sand Darter resulting from the re-establishment of

a population in a formerly occupied Ontario river. Overall, the information provided in the research document can be used by the regional Species at Risk Program to determine if the ecological benefits of an experimental reintroduction outweigh the potential risks for Eastern Sand Darter and the broader ecosystem, and will serve as a case study when considering reintroduction for other SARA-listed species.

Review of Working Paper

Objectives

The objectives, as stated in the Terms of Reference, were elaborated on, noting that an objective in the Recovery Strategy for Eastern Sand Darter (Ontario Populations) was to investigate the feasibility of reintroduction for populations that may be extirpated, and the working paper in this peer review process attempts to evaluate the feasibility to directly support that recovery objective. The authors added that the relative benefit of translocation was greatest when existing populations were declining.

There was a discussion around the definition of “experimental” that was considered here. This could be interpreted as adaptive management, where a reintroduction is undertaken with suitable monitoring, and the approach is adapted as needed; or this could mean a learning exercise. Clarification was needed as to whether this was a true experiment or a recovery measure while applying experimental principles. An author stated they saw less of a divide here, that if the experiment was a success, it also completed a recovery measure, and the primary goal of evaluating the experimental reintroduction was restated. Clarification would be added to the text.

Genetics

A participant raised concerns about identifying an appropriate genetic match, as genetic studies completed to date used neutral genetic markers and were not representative of functional traits. Another participant noted that the usual practice of using the geographically closest population to source individuals for a reintroduction maybe be backward-thinking, given how quickly environmental conditions are changing. It may no longer be suitable to assume that geographically close populations are experiencing shared genetic adaptation, and neutral markers don’t help in our understanding of local adaptations. Another participant noted that it is better to ask which combination of genetic markers will describe the current adaptive potential of possible source populations.

Abundance Estimates (Indices of Abundance)

Responding to written comments about how the abundance estimates for the Grand River were conducted, an author explained some of the assumptions around the resolution of the sidescan sonar work in identifying finer substrate classes (i.e., differentiating sand, silt, and clay), and how the field crews selected random points for sampling (random sites were identified, but only sampled if they contained > 50% sand substrate). A participant felt the assumptions around the sidescan data were problematic, but the resulting estimates seemed reasonable anyways.

A participant cautioned against the use of the term “abundance estimate”, when what was presented was more of an elevated density estimate, and “indices of abundance” was proposed as a better descriptor. There was a discussion around the appropriate fit of the model, where the merits of zero-inflated abundance models, a zero-altered model, and the N-mixture model that was used, were debated. Ultimately, the authors used the N-mixture model given the number of parameters that were included, the multi-state considerations (e.g., related to presence, and presence and at a specific life stage), and the need for predictive extrapolations across areas of the river.

The largest issue raised by a participant about the index of abundance was around the assumption that Eastern Sand Darter density was comparable in wadable (sampled to inform density) and non-wadable habitats (that were not sampled). The participant noted that there could be three options: 1) the density of Eastern Sand Darter could be the same in wadable and non-wadable habitats; 2) density could be greater in wadable than non-wadable habitats (resulting in an over-estimate of index of abundance); or, 3) density could be lower in wadable than non-wadable habitats (resulting in an under-estimate of index of abundance). The participant flagged that there have been a few trawling efforts on the Grand River that would suggest Eastern Sand Darter occupies deeper areas, but we don't know what the true density is in those deeper areas or have enough information to infer amount of suitable habitat available in those deeper areas. The participant suggested identifying the total amount of wadable habitat in the river and then applying the density estimates only to those areas, thus bounding the estimate. An author noted that not only was the amount of wadable habitat important, but the amount of that habitat that contains > 50% sand and fine gravel substrates was also important. The participant agreed, noting the challenges with the available data, but that clarifications to the text regarding assumptions would not suffice; ultimately the participant didn't have confidence in the extrapolations to areas beyond wadable habitats. Another participant elaborated further that the uncertainty and assumptions were acknowledged in the text, but that the real issue was whether the extrapolations in deeper areas are even within the ballpark of reasonable estimates, which was unknown. An approach could be to identify the proportion of habitat expected to be non-wadable vs. wadable, and if the proportion of wadable habitat is high, it would increase the confidence that the abundance estimates are likely reasonable. The authors noted that they were initially comfortable extrapolating the density estimates out to deeper areas because depth was not an important habitat variable in the models. The range of depths used in the models was small (i.e., 0–1.2 m), but so was the range of depths available in the Grand and Thames rivers (i.e., approximately 0–3 m). The authors reiterated their expectation that density was likely similar between wadable and non-wadable habitats based on the available trawling data.

Two possible solutions to this issue were proposed: 1) obtain bathymetric data for the Grand and Thames rivers, determine the total wadable area based on a "wadable" depth threshold (e.g., < 1.2 m), apply the density estimates to those areas, and then apply the density estimates to the rest of the river noting the greater uncertainty in those sections; or, if bathymetric data do not exist, 2) use expert opinion to characterize the proportion of wadable habitat available in each river section. Other participants inquired about whether the sidescan data included depth measurements, but it included depth measurements directly below the boat and not the full bank-to-bank depth profile. The participant agreed that either proposed approach would make the methods more explicit (i.e., what percentage of the habitats match how the data was collected and the model parameterized), but the bigger issue related to whether this affected the decisions that will be made regarding removals causing harm to source populations (i.e., whether they can sustain the removals). The authors responded that this does matter a lot – if we constrain the abundance estimate to only the wadable areas, then the population size likely falls below the minimum viable population (MVP) size threshold, making removals risky for the source population. If you think the proportion of available suitable habitat in the deeper, non-wadable areas is high and densities are similar to wadable habitats, then this matters less (as the abundance estimate based on extrapolated densities is above the MVP).

Bathymetric data were not available, so on the second day, the authors presented tables and figures that described the abundance estimates based on the presumed proportion of wadable habitat and presumed proportion of suitable habitat (based on the proportion of area thought to contain > 50% sand and fine gravel substrates) in each river, and by river section for the Grand River. Some Acoustic Doppler Current Profile data were available to corroborate the expert judgements made in the Thames River about amount of wadable habitat. The abundance estimates still assumed that the density of Eastern Sand Darter was the same in wadable and non-wadable habitats, but the breakdown in the tables would allow participants and managers to sum up the abundance estimates based on their confidence in the proportion of wadable habitat and habitat with suitable substrates. These tables and figures would be provided in the finalized research document. The participant who initially raised this concern would have still preferred to see all three density scenarios modeled, but participants appreciated the revised tables for answering the subsequent questions.

The participant had lingering concerns that there may be two portions of the population that behave differently: a "shallower" subset for which there was a density estimate over presumed suitable habitat, and a "deeper" subset for which there was no density estimate and no idea of suitable habitat availability. An author responded that there was uncertainty built into the model in several places, which yielded a range of abundance estimates, and adding in a second 'group' to the population with a different density estimate would likely just broaden that abundance estimate range slightly, but not greatly. Another participant agreed, stating that whether the density in non-wadable areas was higher or lower than in wadable areas, it would likely just shift where you are within the current range of abundance estimates.

Several participants offered additional observations related to Eastern Sand Darter in deeper habitats to help understand the suitability of extrapolating the densities generated from wadable areas to non-wadable areas. One participant noted that a U.S. study found Eastern Sand Darter on the slope of the thalweg in clear waters, and it might be harder to capture those individuals; another participant noted a similar observation in West Lake, Ontario, that most individuals were collected at the edge of the sand where it drops off to deeper, non-wadable depths; another participant noted that they collected Eastern Sand Darter under overpasses in an Ohio river. Seasonality may be important, as some had detected more Eastern Sand Darter in wadable habitats (with a seine) in the summer, but only detected it in deeper habitats (with a trawl) in the fall.

Other Modelling Considerations

The possibility of sub- or meta-population structure was raised, as there exists a large barrier in the middle of the species' distribution on the Grand River, and the distributions in both the Grand and Thames rivers are very long making it unlikely that Eastern Sand Darter individuals move great distances and mix for reproduction. This has implications for how the MVP threshold should be applied, and for how removals should be conducted. The author team noted that abundance was estimated for each section of the Grand River so MVP thresholds could be applied separately if appropriate, and that the model assumes removals occur across the entire distribution in a source river, so didn't feel there was value in adding in a sub-population component, especially given that an appropriate meta-population structure was not known. Another participant flagged that Eastern Sand Darter abundances were patchy across both rivers, and certain areas were likely to yield very high catches. It would take considerable time and effort to collect individuals from across the distribution in each river. Habitat was especially patchy in the Grand River (e.g., patches of bedrock and other bed materials between patches of sand), where the Thames River had consistently smaller substrate classes throughout. It was agreed that the possibility of sub-population structure should be flagged as an uncertainty, and descriptions of how removals would occur would be more explicit.

An author described how they tried to implement as many scenarios as possible that would restrict success of a reintroduction (e.g., increasing transport mortality) to try to find weaknesses, and almost always ended up with a successful outcome. A participant raised that, because the Ausable River and Big Otter Creek are so large, it's possible that Eastern Sand Darter could move far away from the reintroduction site, limiting interactions with each other (or with individuals released in subsequent years), and therefore increasing the risk of Allee effects and demographic stochasticity. An author reported some results of a tagging study on the Grand River that suggested at least some individuals remained in a relatively small geographic area, and the large penalties (up to 80% mortality) were included to try to consider individuals that may leave the site and not contribute to reproduction. Another participant noted that an exploratory phase post-release in a new environment is common across taxonomic groups, and distance dispersed scales with body size, and will likely relate to patchiness of sand habitats around the release site.

Abiotic Factors

Minor concerns were raised related to the abiotic habitat factors presented in the working paper. The authors responded to written comments regarding habitat matching/equivalency and multivariate statistical approaches that could have been taken. Ultimately, there were too many uncertainties and assumptions with other approaches, but general patterns should be looked at when interpreting the results. A participant inquired about whether dissolved oxygen measurements were taken at the same time of day each day during the Big Otter Creek substrate work, and it was clarified that measurements were taken between 10 am and 2 pm. Another participant asked about the value of, and rationale for, including conductivity and pH in the models. The authors had included those parameters because the data were available and there wasn't a strong case for or against including them. It was agreed the parameters could stay. Participants brought up some habitat work that was done canoeing from Exeter to the Cut on the Ausable River, looking at substrate, depth, and turbidity in riffle-run sequences. Maps were produced from these efforts that generally described the habitat conditions, but data was not thought to be suitable for inclusion here.

Biotic Factors

There was agreement among the group and author team that having multiple years of macroinvertebrate data would be helpful to understand inter-annual variation in prey abundance; however, such data did not exist. Additional potential sources of information were discussed, including possible opportunities to collect these data in the future. One participant flagged additional data that may be available for the Big Otter Creek system; the authors agreed to explore these data and include them if appropriate. The difficulties around answering whether the biotic habitat could truly support a reintroduced population were flagged, noting the lack of precise information on the bioenergetic needs of Eastern Sand Darter and its competitors.

Threats

Participants felt the threats were well covered in the working paper. A participant suggested that future investigation of the density of Eastern Sand Darter and Round Goby in deeper areas (i.e., confirming earlier trawling work in the Grand River) would be beneficial. It was also noted that Eastern Sand Darter was either always present in Big Creek (Long Point Bay) in low numbers, or may have recolonized Big Creek following some abatement of threats related to tobacco farming practices. These abatements have likely led to improved conditions in Big Otter Creek as well. There was discussion highlighting the generally poor knowledge about diseases/parasites/pathogens, both native and novel, in freshwater ecosystems. The disease issue was noted as a global problem not specific to Eastern Sand Darter and there was little to be resolved without broader advances in this research area.

Terminology

Throughout the meeting, several terms were raised that could be misinterpreted if not clearly defined and consistently used. These included: 1) "species" vs. "wildlife species" given the revised designatable unit structure of Eastern Sand Darter and the timing of when that revision would be formalized under the *Species at Risk Act*; 2) "experimental" and the distinction between an adaptive management approach vs. an experiment; and, 3) "populations" and whether this referred to demographic populations of locations/localities where the species is found.

MODIFIED DELPHI – GROUP CONSENSUS QUESTIONS

A modified Delphi approach was used to answer 30 questions related to the probability of success of a reintroduction and the probability of unintended consequences. This approach was intended to be a more quantitative way of gathering expert opinion and capturing associated uncertainty. Following an initial voting round where participants allocated 100 points across 5 categories of certainty, there was a group discussion and then a second round of voting in hopes of arriving at (or closer to) a consensus. A summary of results is presented in Appendix 4. The general approach was described, as was the rationale for using this method. A practice question related to perceptions of participants consuming Canadian-caught freshwater and marine fishes was explored to test out the voting system and practice the vote – discuss – re-vote approach.

Some clarification questions and suggestions were made by participants related to identifying individual participant scores, and maintaining the original voting scores as well as the re-votes. Concerns were raised about how uncertainty would affect the results; if too many people were uncertain, the answers could get washed out. A participant asked about whether individuals have the ability to identify themselves as "not an expert" for particular questions, and opt-out of those questions. The chair and author team emphasized that everyone was invited because of their subject matter expertise. The intention of the exercise was for everyone to take the information from the working paper, supplemented with their own expert knowledge/opinions, and try to answer the question and describe their uncertainty. At the bare minimum, everyone had some baseline knowledge following the review of the research document. The real value was in discussing the scoring/rationale and allowing the group's expertise to be harnessed in a quantitative way. Another participant with experience using this group consensus method urged everyone to resist allocating 20 points across all five categories as much as possible (which would indicate complete uncertainty), but rather to use their judgement based on the information provided to try to answer the questions as best as possible. The authors noted that they could look at how many participants allocated points evenly across the categories for each question as another metric to understand uncertainty. Ultimately, some 'self-policing' would be needed to help understand where the most uncertainty was, but participants were encouraged to think about what questions their personal expertise best addressed, and allocate more points to fewer categories (to indicate higher confidence).

A participant suggested adding a big picture, general question at the beginning and end of the exercise such as "do you think a reintroduction in Big Otter Creek will be successful?" to try to get at the groups overall impression (or gut feeling), before and after the meeting, about the likelihood of success. An author stated this would be difficult to phrase because specific management scenarios were not proposed and the answer may differ depending on the specifics of the scenario. Possible ways of approaching this “big-picture” question were discussed, but ultimately the author team decided not to include it because the overarching objective was to evaluate individual components of the national reintroduction framework, rather than overarching opinions of reintroduction success.

The general process for addressing the questions was as follows: 1) the author team presented the information available (as described in the working paper) to inform that question; 2) the chair opened the discussion for clarification questions to ensure all participants were interpreting the question in the same way; 3) participants opened their respective spreadsheet and allocated 100 points across 5 categories of certainty; 4) the points (votes) were then extracted and graphed; 5) the group was asked for their rationale in scoring (e.g., whether in-line with the majority viewpoint or not), and what other information they considered when allocating their points; 6) votes were re-cast (i.e., points re-allocated) and graphed once again (original and re-votes were retained); 7) changes between the first and second rounds of voting were described and the group was asked to discuss anything that was surprising or unexpected, as needed.

POPULATION

Question 1: What is the probability that population abundance in the Grand River is greater than a) 25,000 Age-1+ individuals; b) 50,000 Age-1+ individuals; c) 100,000 Age-1+ individuals?

Context or clarifications provided: Authors provided revised tables with indices of abundance based on the proportion of wadable/non-wadable habitat, and proportion of suitable habitat. This question was expected to be the hardest to evaluate due to the complexity of the underlying model and extrapolation to wadable and non-wadable areas. Participants were asked to vote.

Round 1 Results (Figure 1a.i): the majority of participants allocated scores in the Very High and High categories, with a moderate number of points in the Medium category, and few points in the lower categories. A participant described how they used the revised table to calculate a reasonable abundance estimate based on their perception of the proportion of suitable habitat in each river section, and placed 80 points in the Very High category, 15 in High, and 5 in Medium. Another participant felt small-bodied freshwater fishes are chronically under-sampled and/or inefficiently sampled, and it is likely that the true density (and therefore abundance) is much higher than what was calculated, so placed all 100 points in the Very high category. Another participant felt unsure about population size, and placed 20 points in each category to express uncertainty, not wanting to influence the overall decision. Other participants noted that the probability categories were not evenly distributed (i.e., Very High was a > 95% probability, while High represented 67–95% probability), and so placed more of their points in the High category rather than Very High because of all the uncertainties and assumptions (they felt sure, but not greater than 95% sure). A participant allocated 90 points to Very High and 10 to High, citing the challenges of sampling small-bodied fishes that occur across patchy habitats and extrapolating out, which likely results in underestimates. They felt very confident that the population was greater than 25,000 individuals, but acknowledged that their certainty would decrease as the potential population size increased. Another participant added that they used their field experience in the river to inform their answer, specifically their knowledge of how many fish can be caught in a 10 m by 10 m area and of the amount of suitable habitat in the river. Participants were asked to re-vote.

Round 2 Results (Figure 1a.ii): there was some shift in points towards Very High and High categories. No further comments were raised.

Participants were asked to vote for question 1 parts b and c simultaneously.

Results Round 1 (Part b); Figure 1b.i): the probability distribution spread out more in b) compared to a). No one allocated 20 points across all categories, three participants placed all 100 points into a single category. A participant offered that they allocated 85 points to Very High and 15 to High for b, based on the abundance estimates from the models presented in the working paper. Another participant said they scored the same as for part a) (80-10-10 in Very High-High-Medium) based on their interpretation of the abundance estimate (being ~ 75,000 individuals).

Results Round 1 (Part c); Figure 1c.i): there was a very different distribution for part c) where most of the votes were placed in the Medium category. Some participants still had most of their points allocated in the Very High and High categories, but most spread their points out across other categories. One participant said they placed no points in the Very High category this time, given the modelled abundances provided (there were few scenarios where the abundance was estimated as > 100,000), so most points were in the High and Medium categories. Another participant with that same rationale allocated points in a similar manner. One participant allocated 75 points to Very High, feeling that the abundance estimates were likely underestimates because current sampling methods are somewhat ineffective at catching small fishes, which justified voting relatively high on this set of questions, though they did allocate more points toward lower categories this time. Another participant added that they spread out their points more than in parts a) or b) than others; they still felt confident the abundance was > 100,000, but less confident than the first two parts. One participant allocated 40 to High, 50 to Medium, 5 to Low and 5 to Very Low, as they didn't have much confidence in the modeled abundances because of detection probability, and not knowing how much suitable habitat is actually available and occupied. They shared that they have done a lot of seining in "suitable" habitat but not captured any individuals. Another participant shared that they shifted more points into lower categories for part c) compared to a) or b); based on the distributions of the N-mixture models, they felt there is a low probability that abundance is > 100,000. It was suggested that there would need to be high densities across a high proportion of suitable habitat for the true abundance to be that high, and as was mentioned by another participant, you often don't catch the species at some of the "suitable" habitats. An author noted that the way the density estimates were generated was meant to account for not all areas of sand and fine gravel being occupied, and that is reflected in the distribution of abundance estimates. Another participant added a reference in the chat related to abundance of an endangered darter from small streams in Florida, where a repeat-sampling approached yielded a density estimate of 500,000 individuals; noting the body size is similar, but the river size was much smaller in the Florida stream, and the estimate was generated using a similar seining approach. Participants were asked to re-vote.

Results Round 2 (parts b and c): slight shift in the results for parts b) and c) – the discussions appeared to give participants a bit more confidence, as they allocated more points in the higher categories than in round 1 (though still less than part a).

Overall: the group was very confident that the true population size was > 25,000, fairly confident it was >50,000, and much less confident that it was > 100,000. No further comments were made.

Question 2: What is the probability that the population in the Grand River would allow the removal of a) 250 Age-1+ individuals; b) 500 Age-1+ individuals; c) 1,000 Age-1+ individuals per year for up to 10 years without reducing the population below $\mathbf{MVP_{99\%}}$ during, and at least three generations after, removal efforts?

Context or clarifications provided: The author team requested that participants approach this question with their assessment of the population size (from Question 1) and evaluate the risk of removals against that population size. An author also clarified that the figures presented showed female-only population size, but the question related to all individuals. A participant asked whether removals could lead to a compensatory increase in the population, and it was clarified that the analysis accounted for this with density-dependent effects. Participants were asked to vote.

Results Round 1 (Figure 2a.i, 2b.i, 2c.i): Part a) resulted in a tiered distribution ranging from Very High to Very Low. One participant noted that they put the majority of their points into the Medium category owing to uncertainty in the modelling dipping below MVP even if no individuals were removed. Another participant shared this concern, placing most of their points in the Very Low category, and reiterated the idea that the dam in the Grand River has likely created two populations, which likely have abundances closer to or below MVP making removals more likely to drop the population below MVP size. A participant asked for clarification around whether the question was referring to just the probability of removals having an impact or if it was the removals plus the fluctuations associated with stochasticity built into the model. An author clarified that it didn't matter because there was no significant effect of the removals causing a dip below the MVP value compared to stochasticity. Another participant described that they simplified the question to thinking that there was a large number of individuals in the system, and this was a relatively small number to remove relative to the MVP, so felt confident that MVP would not be compromised, at least for parts a) and b). Another participant approached this with the belief that the population size is likely ~75,000 (from Q1) and tried to use the figure presented on impacts of removal harm on female abundance, so placed 40 points in each the High and Medium categories and 20 in Low, and applied this across all three parts (i.e., three removal amounts). A participant offered that they felt very confident in part a), allocating 90 points to Very High and 10 to High, but were less certain with b) (80 Very High, 10 High, 10 Medium), and even less so with c) (60 Very High, 20 High, 20 Medium).

An author expressed that this was the general feeling of the group, points were weighted towards the higher categories for part a), but more points shifted to lower probability categories with part b) and again with part c). A participant agreed that their certainty had decreased from parts a) through c), but to a lesser extent than the rest of the group, still allocating most points towards Medium and High, and was mostly concerned about removals from the lower reach of the Grand River where density was lower. Another participant inquired about how the removal values of 250, 500, and 1,000 were determined, expressing concerns that participants may not be thinking of the absolute values, but scoring based on a perceived "low", "medium", and "high" risk, and the vote distributions would look the same regardless of the actual values presented. An author clarified that they tried to arrive at values that seemed logical based on previous permits (of allowed removals) and numbers captured during sampling. The values were not based on a model output, but did not create a significant decline in the population abundance beyond impacts of background noise. There was some discussion among participants around whether collecting 1,000 individuals would be realistic and achievable, and there were questions around how and when removals would occur, which had added uncertainty in the scores. Although removing individuals from across the distribution in a source river would take additional effort compared to concentrating removals at 'hot-spot' patches, participants indicated that it was important to avoid overharvesting hot-spots to avoid jeopardizing survival or recovery. It was also noted that 1,000 individuals was well above what has been permitted for a one-time removal of Species at Risk thus far (450 for Mountain Sucker). Participants were asked to re-vote.

Results Round 2 (Figure 2a.ii, 2b.ii, 2c.ii): more confidence was apparent in the second round with more points allocated in part a) to Very High and High compared to round 1, in part b) Very

High and High retained most of the points, and part c) points were more evenly distributed across the probability categories.

Overall: there was generally high confidence in removing 250 or 500 individuals without compromising MVP, but people were less confident with removing 1,000 individuals.

Question 3: What is the probability that Grand River population-specific life-history characteristics (e.g., age-at-maturity, fecundity, survival, growth, sex ratio) would prevent the establishment of a self-sustaining population?

Context or clarifications provided: Participants asked for clarification that this question means there are no inherent limitations to establishment based on the species life-history, rather than issues like transport-related mortality; the authors confirmed that participants should determine whether life-history parameters that could prevent establishment and development of a self-sustaining population. Some concerns were raised around the sex ratio being unknown, which could lead to negative outcomes by chance. A participant raised that there was no genetic means for identifying the sex of darters at this point, while other participants noted that there may be a few morphological differences, but these would require lethal sampling at worst to validate, or excessive handling at best. Participants were asked to vote.

Results Round 1 (Figure 3.i): most points were placed in the Low category followed by Very Low, but one participant allocated most points to Medium, and another to Very High. One participant indicated that their votes were consistent with the majority (i.e., they felt that the probability of life-history preventing Eastern Sand Darter from establishing was low), but spread a small number of points across other categories as well because there is some uncertainty. Another participant raised concerns that Big Otter Creek is very prone to sedimentation, particularly during flashy storm events, and that individuals sourced from the Grand River (where turbidity is generally lower) may have difficulty adapting to those conditions. An author explained that the Grand River can become quite turbid during storm events as well, particularly in the lower reaches, further supporting the need to source individuals from across the distribution in the source river. Another participant indicated that they allocated most points to Medium because of the concerns around sex ratio and therefore not knowing what has been selected in terms of sex or size when removing individuals. Participants were asked to re-vote.

Results Round 2 (Figure 3.ii): distributions of points shifted minimally following the re-vote.

Question 4: With individuals sourced from the Grand River, a) What is the probability that genetic diversity, variation, and (or) adaptation would prevent a reintroduced population from a) establishing; b) reaching MVP_{99} in Big Otter Creek? And what is the probability that genetic diversity, variation, and (or) adaptation would prevent a reintroduced population from c) establishing; d) reaching MVP_{99} in the Ausable River?

Context or clarifications provided: the question that was originally presented did not identify the recipient river, but it was flagged that answers may differ depending on the recipient river, so the question was re-defined (as worded above). An author suggested it may be helpful to frame this question in terms of an introduced population of 1,000 individuals each year for 10 years. A participant with genetics expertise offered that the genetic information currently available for Eastern Sand Darter is based on neutral genetic markers, which only offers insight on the relatedness of populations and does not confer information about local adaptations. Overall, the number of individuals proposed to be moved was large enough that the risk of a genetic bottleneck would be low (unless extreme levels of mortality occurred). Participants were asked to vote.

Results Round 1 (Figure 4a.i, 4b.i): a participant felt there was nothing to prevent establishment, so they placed most of their points in the Very Low category with a little uncertainty (a few points spread elsewhere). They felt there was still a low probability of anything preventing the population from achieving MVP size, but had slightly less certainty so allocated most points towards Very Low and Low categories. Another participant noted they placed all 100 points in the Very Low category for both a) and b), justified by the large number of individuals released (especially over time) being unlikely to experience a genetic impediment. Another participant placed most points in the Low and Very Low categories, citing some concern around local adaptations from the Grand River being less suitable in a different river. Another participant suggested that local adaptation probably has occurred, but that it seemed to be a relatively minor factor in other stocking programs in terms of species establishment. Subtle differences in outcomes may be observed if survival of differently-sourced individuals was evaluated, but participants agreed that this was likely unimportant here. Participants were asked to re-vote.

Results Round 2 (parts a) and b) Figure 4a.ii, 4b.ii): The distribution of points shifted slightly more towards Very Low and Low in both parts a) and b).

Results Round 1 (parts c) and d); Figure 4c.i, 4d.i): results were similar for the Ausable River and Big Otter Creek (parts a) and b)), with most points being allocated to Very Low and Low for the Ausable River. A participant asked if there were any populations in the Lake Huron drainage that might be more suitable as a source, but the Sydenham River in the Lake St. Clair drainage is the next closest geographically. Given the similarities in responses to part a) and b), the group opted not to re-vote for parts c) and d).

Question 5 : What is the probability that population abundance in the Thames River is greater than a) 25,000 Age-1+ individuals; b) 50,000 Age-1+ individuals; c) than 100,000 Age-1+ individuals?

Context or clarifications provided: This question was similar to Question 1, but focused on the Thames River as the source location. Authors provided revised tables with indices of abundance broken down by proportions of wadable/non-wadable habitat, and proportions of suitable habitat. There was a brief discussion around water depths in the Thames River, noting that there was more wadable habitat there than in the Grand River, which increased confidence in the indices of abundance. It was also noted that the data presented for the Thames River were based on fish sampling from 2006. Participants were asked to vote.

Results Round 1 (Figure 5a.i, 5b.i, 5c.i): results were very similar to the Grand River in Question 1, where most points were allocated to Very High for part a), some participants shifted to lower categories for part b), and even more participants shifted towards lower categories for part c). One participant noted that individuals were smaller in the Thames River, and owing to the turbidity, they likely live a shorter, tougher life compared to the Grand River. This participant believed that the number of age-1+ individuals is less in the Thames River compared to the Grand River. They still distributed most points to higher categories, but with more spread (less confidence) than for the Grand River. Another participant added that their scores and the progression towards more spread from parts a) through c) aligned with how the rest of the group voted; however, they were surprised the group had so much confidence in parts a) and b) given that the probability distributions (i.e., index of abundance table) suggested a fairly high number of outcomes were less than MVP size. Another participant shared this concern, stating that based on the probability distributions, 50% of the time the population size would be less than 100,000. A participant stated that they assumed the proportion of suitable habitat was 20–25% to inform their abundance estimate, providing high confidence for part a) but low confidence for parts b) and c). They also noted that Round Goby has been present in the system for almost 20 years since the data informing the density estimates were collected, creating uncertainty in the understanding of present-day population abundance. Another participant agreed they had less confidence for part c) compared to parts a) or b), and felt there were more uncertainties because of the higher turbidity in the Thames River compared to the Grand River, which could lead to reduced detection probability. Participants were asked to re-vote.

Results Round 2 (Figure 5a.ii, 5b.ii, 5c.ii): the distributions of results were similar to the first round, but with slightly less confidence for part a), less confidence for part b), and much less confidence for part c). No additional comments were raised.

Question 6: What is the probability that the population in the Thames River would allow the removal of a) 250 Age-1+ individuals; b) 500 Age-1+ individuals; c) 1,000 Age-1+ individuals per year for up to 10 years without reducing the population below $\mathbf{MVP_{99\%}}$ during, and at least three generations after, removal efforts?

Context or clarifications provided: This question was similar to Question 2, but for the Thames River as the source location. The authors again suggested that participants approach this question with their understanding of what the population size is (from Question 5) and evaluate the probability of removals against that, noting that the Thames River indices of abundance were lower than for the Grand River. Participants were asked to vote.

Results Round 1 (Figure 6a.i, 6.i, 6c.i): there was generally most support for Very High and High in part a), then a general decrease in certainty as the group moved through parts b) then c), as with the Grand River. A participant offered that they used the figure of impact of harm on female abundance to answer this question, noting that the relationship was a relatively flat line (indicating little impact of harm from removals), but felt that the population size in the Thames River was too low to sustain removals, so scored 40-20-20 in Medium, Low, and Very Low categories, respectively, across all three removal scenarios. Another participant noted that the models assume that the population growth rate was stable, but they felt uncertain about that assumption so allocated more points to the lower categories, especially for part c). Another participant stated that they felt that the species was likely under-sampled and therefore more abundant than the models suggest, so this reduces the risk in their mind, at least over a 10-year timeframe. There was a discussion among participants regarding whether removals were in addition to natural mortality and what the growth rate was thought to be. If the population is at carrying capacity, then removals may allow for population growth by relaxing the density-dependent effects; however, if the population is not at carrying capacity and is limited by unknown factors (e.g., threats), then removals do not relax the density dependence and removals could lead to further declines. Not knowing the true population growth rate added uncertainty around the impact of removals. Participants were asked to re-vote.

Results Round 2 (Figure 6a.ii, 6.ii, 6c.ii): the distributions were similar to Round 1, with a slight shift in points towards higher categories for b) and a slight shift towards lower categories for part c). A participant considered, much like for the Grand River, whether the results were more driven by a perceived level of risk associated with the three removal scenarios, rather than the absolute values, and if the distributions would look the same if a different set of “small”, “medium”, and “large” removal values were presented. Another participant noted that they carefully considered the actual values, where scenario c) would mean 10,000 individuals were removed over 10 years and that might result in some compensatory response. A participant offered that they had a similar response for all three scenarios, as in Round 1, driven by the fact that the Thames River population size wasn’t likely very large overall and even 250 individuals (part a) seemed like a lot.

Question 7: What is the probability that Thames River population-specific life-history characteristics (e.g., age-at-maturity, fecundity, survival, growth, sex ratio) would prevent the establishment of a self-sustaining population?

Context or clarifications provided: This question was similar to Question 3, but for the Thames River as the source location. Participants were asked to vote.

Results Round 1 (Figure 7.i): the distribution was skewed towards Low and Very Low. A participant noted that it made sense that there was greater uncertainty here than with the Grand River, given that there were more small individuals. There was discussion around whether the size distribution could be a consequence of rearing environment, and if individuals were moved to a less stressful environment, those growth constraints could be relaxed. Other participants agreed with this logic, citing this growth difference as the reason for less confidence in their votes compared to the Grand River (shifting more points from Medium to Low). A participant offered that food availability and limits to food acquisition associated with higher turbidity could be why smaller individuals exist in the Thames River, and that this could limit success as, ideally, large females would be transferred. A participant asked for clarification around whether the Thames River fish don't live as long, or simply don't grow as much, which was unknown with available data. It was reiterated that smaller individuals may be less fecund, which could limit success, but there could be other limitations to using smaller individuals as well. It was confirmed that the timeframe being considered for this question was just related to establishing a self-sustaining population, noting that achieving an MVP-sized population would take longer. Participants were asked to re-vote.

Results Round 2 (Figure 7.ii): the distribution was similar to Round 1 with a slight shift of points towards Medium and less in the Low category. A few participants added that they shifted more points to Medium because of uncertainties around the body size of Thames River individuals.

Question 8: With individuals sourced from the Thames River, a) What is the probability that genetic diversity, variation, and (or) adaptation would prevent a reintroduced population from a) establishing; b) reaching MVP_{99} in Big Otter Creek? And what is the probability that genetic diversity, variation, and (or) adaptation would prevent a reintroduced population from c) establishing; d) reaching MVP_{99} in the Ausable River?

Context or clarifications provided: This question was similar to Question 4, but for the Thames River as the source location. Participants were asked to vote.

Results Round 1 (Figure 8a.i, 8b.i): Big Otter Creek part a) results were skewed towards the low categories. A participant stated that they scored this the same as for the Grand River as they didn't see strong reasons for expecting a difference between the two rivers. Another participant suggested there was likely genetic variability that can't be ignored. A participant offered that they placed some points in the Very High category, because of uncertainties around adaptation potential. Adaptation potential is known across populations of species that are used in stocking programs, but those species are typically good at colonizing new habitat; data are limited on habitat-restricted species like Eastern Sand Darter, but fitness consequences have occurred in other species (e.g., Slimy Sculpin *Cottus cognatus*). Another participant shared that they moved some points to the High category to reflect their uncertainty, but noted examples of where Eastern Sand Darter has recolonized habitats from which it was formerly extirpated in the U.S. Part b) had a very similar distribution. Participants were asked to re-vote.

Results Round 2 (Figure 8a.ii, 8b.ii): no major changes were observed.

Results Round 1 (Figure 8c.i, 8d.i): Ausable River part c) results were very similar to Big Otter Creek, which is the same pattern that emerged for the Grand River as well. Participants were asked to re-vote.

Results Round 2 (Figure 8c.ii, 8d.ii): no major changes were observed.

HABITAT

Question 9: What is the probability that suitable abiotic conditions for Eastern Sand Darter in the Ausable River are a) available; b) available in sufficient quantity to support 5,000 individuals; c) available in sufficient quantity to support a population abundance equal to MVP_{99}?

Context or clarifications provided: the authors added that they chose 5,000 for part b) as an intermediary value between 0 and MVP size; it was a value they felt was useful in an experimental context. The authors provided a table of occupancy modelling results in extirpated waterbodies from a previous publication (Dextrase 2013) to help inform their decisions. A participant inquired as to whether this question was related to quality or just quantity of habitat. The authors clarified that part a) was related just to quality, but parts b) and c) incorporate information about quality and quantity. Information on quantity of total available habitat in the Ausable River and Big Otter Creek from the working paper was reiterated to the group. A lengthy discussion around appropriate thresholds for measuring success ensued. Participants raised concerns around the MVP_{99} threshold being overly conservative, and if that extremely high bar was the threshold, it likely couldn't be met, and reintroductions would never be undertaken. Another participant explained that MVP thresholds need to identify a risk level and a time horizon (as was done in the current estimate) and that the time horizon chosen by the author team aligns with how the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) evaluates probability of persistence. The authors justified including this 'high bar' (in addition to the more moderate 5,000 threshold) because if the higher threshold can be met, then managers may be more willing to proceed with the reintroduction. There was discussion around risk tolerance of different stakeholders and how that may be dictated by their responsibilities (e.g., government agencies with a mandate vs. academics with experimental interests). An author added that the choice of timeframe, catastrophe rate, and even quasi-extinction thresholds were fairly conservative and, in some cases, arbitrary, because of uncertainties, but ultimately a value needed to be chosen, so they selected the median MVP estimate as a reasonable value for a future population size. Another participant suggested viewing this from an experimental lens; a successful management outcome might mean a long-term self-sustaining population of MVP size, but a successful experimental outcome might mean a self-sustaining population of a few thousand individuals over a shorter time frame. Other potential measures of success, such as overwinter survival, reproduction, and recruitment, were mentioned as possible criteria for success if the experimental reintroduction is undertaken. A participant added a final consideration around stochastic events, like a manure spill from a pig farm resulting in extremely high local mortalities of another SAR fish. Participants were asked to vote.

Results Round 1 (Figure 9a.i, 9b.i, 9c.i): in part a), points were allocated mostly towards the High and Very High categories suggesting participants felt that the appropriate abiotic factors were available; in part b), many points moved to Medium, but were still skewed towards the higher categories; and in part c) most points were allocated to the Medium category. One participant offered that they allocated 100 points to Very High for part a) as it was likely that there was at least one area with suitable habitat within the stretch of the Ausable River under consideration. Another participant allocated most points to Medium for all three parts of the question, citing many uncertainties, differences in hydrology, and abiotic features that were not accounted for. A participant considered the most important variables to be percent composition of sand and dissolved oxygen, and noted that the mean values of these measured variables tended to be lower (with tighter standard deviations) in the Ausable River compared to the Grand or Thames rivers but still seemed reasonable, so scored 70 points in the Very High category and the remaining points were spread to lower probability categories. Another participant agreed with the logic that abiotic parameter values were generally lower (worse), specifically more silt and less sand, in the Ausable River compared to the Grand or Thames so placed more points in the middle categories. Further clarification was added that part a) considers whether suitable habitat is available somewhere in the system, but doesn't necessarily need to support a large number of Eastern Sand Darter. Other participants agreed that the lower reaches of the Ausable River were generally poorly suited to Eastern Sand Darter because of low flows and siltation, but the upper reaches contain areas of clean sand (i.e., between Exeter and Ailsa Craig). A participant added that they had allocated 20 points across all categories for part c) because of limited local knowledge of the system and were grateful to hear descriptions from those with local knowledge. Participants were asked to re-vote.

Results Round 2 (Figure 9a.ii, 9b.ii, 9c.ii): part a) saw a shift from Very High to High, part b) a shift from Very High to Medium, and part c) a slight shift towards Low. One participant added that they had originally scored 50 points in the Very High category for part b), but they had scored lower in the second round because they had little confidence in there being suitable habitat at one site (i.e., part a), so had even less confidence in there being enough habitat to support 5,000 individuals.

Question 10: What is the probability that there is sufficient connectivity between habitats in the Ausable River to support all life-stages of a reintroduced population?

Context or clarifications provided: connectivity issues in the Grand River (dams as barriers and patchiness of sand substrates/depositional zones) were briefly discussed, and participants were urged to think about the likely home range of Eastern Sand Darter when considering habitat connectivity. A participant asked if uncertainty of survival (considered in earlier questions) should be factored into their answers here, or if they were to assume survival will occur if connectivity is good; the authors responded that the latter was correct. Participants were asked to vote.

Results Round 1 (Figure 10.i): most points were allocated to the Very High and Medium categories. A participant asked whether they should consider a "reintroduced population" as a sustainable population or just an individual breeding pair; it was determined that just a breeding pair completing their cycle could be considered. They added that the larval drift phase is relatively short and the size difference between the drift phase and when individuals become benthic is only a few millimeters, so small areas are needed to support the small amount of growth to become benthic, and thus they allocated most points to the High category. A participant added that they felt they didn't have enough information regarding spatial distribution of suitable habitat (or patchiness) in the Ausable River to properly evaluate this question, so spread points out across probability categories. Another participant shared similar concerns about the scale of connectivity, acknowledging that the upper section was described as suitable (or likely to contain suitable habitat) and the lower section as likely not suitable, so the importance of connectivity might differ between those sections. Another participant offered that they had been thinking about spatial configuration of suitable patches more in Question 9, and was thinking about artificial barriers for this question, so allocated most points to the Very High category as they were not aware of any constraints here. Participants were asked to re-vote.

Results Round 2 (Figure 10.ii): responses were almost identical to Round 1.

Question 11: What is the probability that suitable abiotic conditions for Eastern Sand Darter in Big Otter Creek are a) available; b) available in sufficient quantity to support 5,000 individuals; c) available in sufficient quantity to support a population abundance equal to MVP_{99}?

Context or clarifications provided: This question was similar to Question 9 but for Big Otter Creek as the recipient location. The authors added that there had been some targeted habitat sampling in Big Otter Creek in 2018, so more information was available here than for the Ausable River. Participants were asked to vote.

Results Round 1 (Figure 11a.i, 11b.i, 11c.i): A participant with local knowledge of Big Otter Creek indicated that they felt the habitat was great because of all the sand, but there is a high volume of silt that gets deposited during high flow conditions. This silt creates a thick, slimy film on the creek bed following settling. Other participants noted that this happens on the Thames River in periods of high flow as well (although it was uncertain if clay or silt was causing the issue). Another participant stated that they allocated most points to the Very High and High categories for all three parts of this question based on the occupancy models in Dextrase (2013), where occupancy was anticipated to be higher in Big Otter Creek compared to the Ausable River, and from the targeted habitat sampling from two studies that found high amounts of sand substrates. This participant flagged a concern around the thermal regimes, as there is more groundwater in Big Otter Creek compared to the Grand or Thames rivers, but this was also true before the species was extirpated and the temperature signature likely has not changed greatly since that time. There was a brief discussion around why temperature might be limiting (it could lead to slower first year growth and reduced overwinter survival), but this might be a distinction between suitable and productive habitat. It was raised that Big Creek also has cooler temperatures (likely cooler than Big Otter Creek) and supports fewer Eastern Sand Darter, but that this groundwater may actually provide a buffer against future climate warming. Another participant offered that they also allocated most points to the Very High and High categories based on the information presented in the working paper. Another participant offered that tobacco farming was the likely reason why the species disappeared from both Big Otter Creek and Big Creek, and due to improved agricultural practices, the species has re-colonized Big Creek. They felt the same potential existed for Big Otter Creek (offering greater certainty than in the Ausable River where the Cut was still impacting sediment transport and flows). Participants were asked to re-vote.

Results Round 2 (Figure 11a.ii, 11b.ii, 11c.ii): very little change was observed for part a), there was a shift towards Very High and High in parts b) and c) compared to round 1, as the discussion gave people more certainty.

Question 12: What is the probability that there is sufficient connectivity between habitats in Big Otter Creek to support all life-stages of a reintroduced population?

Context or clarifications provided: This question was similar to Question 10, but for Big Otter Creek as the recipient location. There is a dam on the mainstem of the creek, but it is located upstream of the reach of interest for a reintroduction. Participants were asked to only consider barriers, and not consider any particular population size. Participants were asked to vote.

Results Round 1 (Figure 12.i): most points were allocated to the Very High and High categories. One participant offered that they copied their scores from Question 10 for the Ausable River here considering similarities in placement of dams (upstream of the reach of interest). Other participants used similar scoring and logic based on the location and number of known dams, and others noted the relatively consistent sand substrate in this creek, suggesting patch connectivity is high. Participants were asked to re-vote.

Results Round 2 (Figure 12.ii): there was a slight shift towards Very High and fewer points in Medium.

Question 13: What is the probability that suitable food resources for Eastern Sand Darter in the Ausable River are a) available; b) available in sufficient quantity to support 5,000 individuals; or, c) available in sufficient quantity to support a population abundance equal to MVP_{99}?

Context or clarifications provided: the structure of this question was similar to Question 9 on abiotic conditions. The authors noted that there was a limited amount of data on seasonal and annual abundances of benthic macroinvertebrates. Participants were asked to vote.

Results Round 1 (Figure 13a.i, 13b.i, 13c.i): part a) showed most points allocated to the Very High and High categories. A participant offered that they allocated more points to Medium because of the uncertainty in food availability. Another participant allocated most points to Very High and High for part a) as they felt confident food was available somewhere in the system, but shifted points to lower probability categories in parts b) and again in c) because of the uncertainty in total availability. Another participant shared this logic, but placed most points for part a) in High and Medium, shifting points to lower categories for part b), and allocated 20 points to each category for part c) indicating a lack of certainty. Other participants echoed that they shifted points to lower probability categories progressing from parts a) through c). A participant offered that they felt uncertain about how much food Eastern Sand Darter individuals need, in addition to what is available. Another participant allocated 5 points to Very High, 80 points to High, and 15 to Medium for parts a) and b) because most of the macroinvertebrate species consumed by Eastern Sand Darter are fairly ubiquitous and effective dispersers, and 5,000 Eastern Sand Darter is not a lot of fish for the system to support; however, they felt less confident for part c). Another participant felt that southwestern Ontario systems are relatively productive, so they weren't too concerned from an energy availability perspective (for either the Ausable River or Big Otter Creek), but noted that their scores did follow the same pattern as others, with points shifting towards lower categories in parts b) and again in c). They further added that a modelled solution would be useful for answering c), given the MVP was a modelled value. A participant asked if this should be thought of as food availability in the absence of other species that may also be using those resources, or food availability given that competition is occurring. An author responded that this should consider food that is available to the species (factoring in competition). Another participant shared that they allocated most points to the Medium, Low, and even Very Low categories, especially for part c) (given the earlier discussion around MVP being a high bar); they did not necessarily feel that there was not enough food, but their scores reflected the uncertainty, especially given environmental stochasticity. Participants were asked to re-vote.

Results Round 2 (Figure 13a.ii, 13b.ii, 13c.ii): part a) stayed roughly the same as round 1, but part b) showed a shift of points from Very High towards High and Medium, and c) to Medium and Low categories.

Question 14: What is the probability that suitable food resources for Eastern Sand Darter in Big Otter Creek are a) available; b) available in sufficient quantity to support 5,000 individuals; or, c) available in sufficient quantity to support a population abundance equal to MVP_{99}?

Context or clarifications provided: this question was similar to Question 13, but for Big Otter Creek as the recipient location. The authors noted that there was some benthic macroinvertebrate sampling conducted on Big Otter Creek to help inform this question, but it was limited in scope. The data were reflective of relative abundance and not total abundance due to subsampling methodology. A participant indicated a belief that Big Otter Creek is a less productive system than the Ausable River likely because of reduced phosphorus loading in Big Otter Creek in recent years. Another participant stated that historical sampling efforts of benthic macroinvertebrates in Big Otter Creek suggested there were a lot of chironomids at Calton, and fewer but still many at Black Bridge, and added that the constantly shifting sand bedload may cause shifts in the benthic macroinvertebrate community. Participants were asked to vote.

Results Round 1 (Figure 14a.i, 14b.i, 14c.i): part a) had most points allocated to the Very High and High categories; points shifted towards the High and Medium categories for part b), while most points were allocated toward low, medium, and high for part c), with some points in Very High and Very Low, reflecting more uncertainty. A participant shared they used the same scores as for the Ausable River – they felt fairly confident for part a) so allocated more points to the higher categories, but were less confident for part b) so shifted points lower, and then allocated 20 points to all categories for part c). Another participant felt less confident that Big Otter Creek could support Eastern Sand Darter compared to the Ausable River, based on their experiences with both systems and observing more diversity in the Ausable River. Another participant also felt less certain about Big Otter Creek compared to the Ausable River citing the benthic invertebrate data, which showed that there was less diversity overall and some prey groups missing from the Big Otter Creek samples. An author added that it was difficult to compare the Ausable River and Big Otter Creek because there were more data for the Ausable River (the authors were not previously aware of the existence of Big Otter Creek historical data), and there is substantial variation in the availability of data through space and time for each location. A participant asked if the data were collected across a variety of substrate types, and it was explained that benthic invertebrate data were collected annually at index stations on the Ausable River that aim to cover riffle, run, and pool habitat types (thus would not target sand specifically), while data collected in Big Otter Creek was collected at sites historically occupied by Eastern Sand Darter or adjacent to those sites where sand banks occurred. It was pointed out that more stable substrates typically support higher diversity of benthic macroinvertebrates, so if samples were collected only over sand, less diversity would be expected. Another participant added that they were less concerned about whether the appropriate taxa were present, but more concerned about issues around system productivity that others had raised, so they allocated more points to the lower categories to reflect added uncertainty compared to the Ausable River. A participant felt confident there was food (higher score for part a)), but as the number of Eastern Sand Darter increased in parts b) and c), there would be increased intraspecific competition in addition to interspecific competition with Round Goby and other benthic fishes; Eastern Sand Darter tend to have reduced body condition in areas where they overlap with Round Goby. Participants were asked to re-vote.

Results Round 2 (Figure 14a.ii, 14b.ii, 14c.ii): there was little change for part a), a slight shift from High to Medium for part b) and a further shift to lower categories for part c).

Question 15: What is the probability that a) competition; or b) predation would prevent the establishment of Eastern Sand Darter during 10 years of reintroduction efforts in the Ausable River?

Context or clarifications provided: information presented in the working paper was reiterated regarding the number of fish species, and the number of assumed predators and competitors for the Ausable, Grand, and Thames rivers. This did not consider relative abundance, just number of species present (comparable across river systems), and some relationships were assumed based on ecology but lacked quantitative analyses. A participant inquired about what was known about interactions with other darter species, or other benthic species; some studies suggested the potential for a negative interaction with Rainbow Darter (*Etheostoma* *ceruleanum*) but this was likely reflective of habitat partitioning. Another participant offered that they had investigated biotic factors in occupancy models, and found that trophic groups played a role in the additive models, but it was never a primary factor influencing occupancy or abundance. Other two-species co-occurrence models found evidence of other negative interactions with smaller benthic or pelagic species. Participants asked if they were meant to consider interactions with Round Goby in this question, given that it was also considered later on in the threat-related questions. The authors responded that, hypothetically, Round Goby could be removed from a system if the level of threat was thought to be significant enough, but that would never be considered for native species, so best to evaluate it independently and think only of native species in this question. Another participant asked if fish community data are available from historically occupied sites on the Ausable River and Big Otter Creek to compare to the community data on the Grand and Thames rivers to identify potentially novel species in the reintroduced sites that they did not evolve with at the source locations. Additional fish data from recent targeted sampling in the Ausable River and Big Otter Creek were shared, flagging that the recent sampling on the Ausable River occurred upstream of the historically occupied sites. Another participant suggested that the diet overlap study on the Sydenham River (Firth et al. 2021) could also help inform competition. The relevance of avian predators was briefly discussed, but it was assumed to be of minor importance given the turbidity in the Ausable River and the cryptic colouration of Eastern Sand Darter, and few water birds in the upper reaches.

Results Round 1 (Figure 15a.i, 15b.i): most points were allocated to lower categories for part a) (competition), and more points in the Medium and Low categories for part b) (predation). A participant allocated 10 points to Medium, 10 to Low, and 80 to Very Low given that the fish communities in the Grand and Thames rivers appear similar to the Ausable River, with some uncertainty related to relative abundance patterns. Another participant agreed with this logic, citing similarities in the source rivers to the Ausable River fish community and that Eastern Sand Darter is already living with those same species in places where it is doing well; they placed most points in Medium and Low categories. A participant inquired about likely changes in the fish community since the historical record of Eastern Sand Darter in the Ausable River. Others thought that some shifts may have occurred but no great differences, and that the fish community has remained relatively stable in at least 18 years. Another participant stated that they had less certainty about this question and allocated points more widely across categories because Eastern Sand Darter has not been present in the Ausable River (or Big Otter Creek) for many decades, so their food resources may be used by other species. Those other species are likely to be at carrying capacity, so reintroducing Eastern Sand Darter may increase competition relative to where it already exists. Another participant agreed with this logic about Eastern Sand Darter having to compete to 'get back in' to the community, so allocated 50 points to Medium and spread the remaining points around the medium category. Another participant felt that the risk of competition was likely low, given the specialized niche of Eastern Sand Darter. There have been other cases of successful reintroductions of darters and madtoms with no apparent signs of competitive interactions that limited establishment. There were concerns around reintroducing Redside Dace (*Clinostomus elongatus*) in streams that contained large-bodied predators like Northern Pike (*Esox lucius*) and Brown Trout (*Salmo trutta*), but there were no similar concerns here. Additionally, Eastern Sand Darter may be able to evade predators with its fossorial behaviour. Participants were asked to re-vote.

Results Round 2 (Figure 15a.ii, 15b.ii): there was little change in responses compared to round 1. A participant asked the rest of the group what the logic was for some scores in the Very High and High categories. A participant added that they allocated 10 points to Very High because initial predation rates can be very high following stocking events, and others shared this logic.

Another participant suggested there are some options for reducing predation on newly released individuals.

Question 16: What is the probability that a) competition; or b) predation would prevent the establishment of Eastern Sand Darter during 10 years of reintroduction efforts in Big Otter Creek?

Context or clarifications provided: this question was similar to Question 15, but for Big Otter Creek as the recipient location. As before, the number of presumed predators and competitors known from this system was presented and compared to the Grand and Thames rivers. Participants were asked to vote.

Results Round 1 (Figure 16a.i, 16b.i): most points were allocated to the Medium and Low categories in both parts a) and b). A participant stated that they used the same scores as for the Ausable River as the fish communities are similar, but did allocate a few points to Very High for b) because of examples of Brown Trout and Redside Dace predation mentioned previously. If it so happens that a predator is nearby when Eastern Sand Darter is released, it could be catastrophic. Another participant also used the same scores as for the Ausable River. Participants were asked to re-vote.

Results Round 2 (Figure 16a.ii, 16b.ii): results were nearly identical to Round 1.

THREATS

Question 17: What is the probability that agricultural activities would prevent the establishment of an Eastern Sand Darter population across 10 years of reintroduction efforts in the Ausable River?

Context or clarifications provided: the amount of agricultural land use in the Ausable River watershed was compared to that of the Grand and Thames river watersheds; data did not exist to describe the types of agricultural land use (e.g., cattle, row crops). There were no major trends through time in water quality related to agricultural land use. A participant asked if nutrient data were included, as differences between the Ausable and Grand/Thames rivers could be informative. A participant asked for additional context from those with local knowledge of the Ausable River and Big Otter Creek watersheds related to historical tobacco farming, and current levels of cattle grazing and riparian buffers. One participant responded that they believed the Ausable River had less riparian buffer compared to Big Otter Creek (based on information contained within watershed report cards), and that there is a lot of row crop agriculture and tile drainage in the Ausable River watershed, but little cattle grazing. Participants noted that practices have improved in recent years, with more cover crops and wetland creation. Another participant flagged that most of the issues being discussed related to chronic stressors, but that spills (e.g., manure, fertilizer, chlorine, pickle brine) have occurred in Ontario resulting in large fish mortalities. A participant added that fish kills have occurred, but in smaller tributaries and drains rather than the main channel where Eastern Sand Darter would be reintroduced. Context from Big Otter Creek was also raised: historical tobacco farming was very hard on the soils, but has mostly shifted to cash crops (e.g., corn, soy) but with fruit and ginseng farms being common as well. Dairy and livestock production are common upstream, and there has been little uptake from farms in maintaining livestock exclusion fencing or riparian buffers, but the relatively large groundwater inputs alleviate some of these upstream issues. A participant inquired about whether any agricultural best practices or management activities would occur alongside reintroductions of Eastern Sand Darter to ensure that practices don’t change over the next 10 years and lead to further degradation. It was stated that if there was information to suggest that practices could change, then it should be considered, but there wasn’t such evidence. A participant added that they felt 10 years was too long of a timeframe to consider threat impacts, and that the deposition of fines associated with agricultural activities is likely a greater concern for the Ausable River compared to Big Otter Creek because of the surficial geology being more clay in the former and more sand plains in the latter. It was also raised that requests and approvals for enclosing headwater streams and agricultural drains seem to be increasing in recent years, and this combined with tile drains has led to flashier systems compared to historical conditions. Participants were asked to vote.

Results Round 1 (Figure 17.i): Most points were allocated to Medium and, to a lesser extent, Low. A participant shared their surprise that the points were skewed to Medium and Low categories, as they had placed more points in the higher categories, citing limited evidence that agricultural practices have improved greatly over the last several decades and that is considered the primary cause of the species' extirpation from three historically occupied localities. They further added that farm equipment has gotten bigger, and buffers and treed property lines have been removed in favour of creating a single, larger field. Another participant indicated that they allocated most points to Medium and Low (aligning with the majority) because the species is persisting in other areas with intensive agricultural land use and there are some improvements (notably in the Ausable River) compared to historical conditions. However, this participant placed some additional points in the Very Low and Very High category to reflect uncertainty especially due to increasing enclosures. Another participant asked if tobacco farming historically occurred in the Ausable River watershed, but this was not thought to be the case. The especially intensive practices of tobacco farming (high proportion of exposed sand, substantial water extraction) were thought to be the primary drivers of extirpation from Big Otter Creek. If more standard agricultural practices were employed in the Ausable River watershed, then there are likely other drivers that led to its extirpation in the Ausable River and not in the Thames River. Another participant allocated points with most centered on Medium. Participants were asked to re-vote.

Results Round 2 (Figure 17.ii): results were nearly identical to Round 1.

Question 18: What is the probability that Round Goby would prevent the establishment of an Eastern Sand Darter population across 10 years of reintroduction efforts in the Ausable River?

Context or clarifications provided: literature related to Round Goby and small native benthic fishes was briefly reviewed. Additional unpublished work (K. McAllister, U. Waterloo) looking at isotopic diet shifts of other darter species as a result of Round Goby invasion was described (changes in diet related to spatial distribution and relative abundance as opposed to before/after invasion). Work investigating impacts of Round Goby on Channel Darter (*Percina copelandi*) was discussed, noting that an initial decrease in Channel Darter abundance was observed in the Trent River, but that the species has persisted despite Round Goby invasion. Lastly, sampling in the Sydenham River at index stations over the last 10–15 years suggest a decrease in Eastern Sand Darter as Round Goby increased and moved upstream. Participants were asked to vote.

Results Round 1 (Figure 18.i): Most points were allocated to the Medium category, with fewer points allocated to Low and High, and even fewer to Very Low and Very High. One participant offered that they allocated most points to Medium, with some in Low then Very Low. This was not because Round Goby isn't important, but that it currently has a relatively restricted range in the Ausable River that is well below the reach where reintroductions would likely occur. They also noted their concern about potential declines of Eastern Sand Darter in the Sydenham River related to Round Goby, but cited more prevalent cobble substrate driving abundance of Round Goby in that system, which would be less of a concern in the Ausable River. A participant emphasized the need for relative abundance data in addition to occurrence data, as abundances are generally lower (as are impacts) at the invasion front. Round Goby is agonistic and aggressive towards other benthic species. Another participant allocated most points to Medium but did allocate some points across all other categories because of uncertainty in the spread of Round Goby over the next 10 years. Another participant tried to partition the risk of negative impacts, the probability of spatial overlap, and whether sufficient numbers exist for negative impacts, and stacking all of these probabilities resulted in a down-weighting of the scoring. Overall, they felt the risk of Round Goby preventing establishment of Eastern Sand Darter was low. A participant allocated most points to the Medium category with some points added to other categories, and felt that timing was key; if Eastern Sand Darter could establish before Round Goby spreads and takes hold in the Ausable River then the chances of success are higher, but if Round Goby takes hold first, it could preclude Eastern Sand Darter. A participant asked for additional context on the invasion front in the Ausable River, expressing concern that the data presented were eight years old, and Round Goby could have increased its distribution and abundance in that time. Sampling at index stations occurs every summer, and most recently it was detected at Sylvan (as described in the working paper), but not in Ailsa Craig. The rate of spread in other rivers was not explicitly known, but some participants thought it could be related to flow. Another participant noted that Round Goby is relatively abundant (based on benthic trawl data) at, and downstream of, the Cut area, which is mostly at lake elevation. On the Grand River, the invasion front seemed to shift a few kilometers up and down either side of an oxbow per year, depending on flow conditions. Participants added context around Round Goby being detected in reservoirs along the Grand River and Big Otter Creek, which implies that secondary spread and probable human-mediated dispersal has contributed to its distribution in those systems. There was agreement around the difficulties in scoring this as many factors contribute to the perceived influence of Round Goby on the establishment of Eastern Sand Darter. Participants were asked to re-vote.

Results Round 2 (Figure 18.ii): there was a slight shift in points from Medium towards Low.

Question 19: What is the probability that pathogens or parasites would prevent the establishment of an Eastern Sand Darter population across 10 years of reintroduction efforts in the Ausable River?

Context or clarifications provided: The group discussed the limited knowledge and baseline data available on pathogens and parasites for freshwater fishes in general. The authors assumed that diseases are likely similar between source and recipient locations in Southwestern Ontario. A participant agreed with this logic based on biogeography, at least for native pathogens, feeling it was unlikely that a pathogen would be found in one watershed (Grand or Thames rivers) but not in another (Ausable River or Big Otter Creek). The participant noted, however, that most agencies are not screening for diseases in Ontario except for viral hemorrhagic septicemia (VHS). Novel (non-native) diseases remain an unknown, particularly for sub-lethal effects, but there is limited agency capacity and expertise to undertake this work in Ontario. A potential study design that could address these gaps was briefly mentioned. A participant shared insights from colleagues at a disease center regarding how important disease was for fitness effects, and how disease may affect reintroduction success, but limited information existed to link these with source and recipient populations of Eastern Sand Darter. Participants were asked to vote.

Results Round 1 (Figure 19.i): most points were allocated to Low, Medium and Very Low; however, several participants allocated 20 points to each category indicating their overall uncertainty about disease issues. One participant flagged that this is likely to be one of the greatest uncertainties relating to reintroductions that requires research investment, and may represent one of the greatest risks of reintroduction. Some additional resources from the US

Fish and Wildlife Service Fish Health Center and DFO's National Aquatic Animal Health Laboratory System were provided in the chat. Participants were asked to re-vote.

Results Round 2 (Figure 19.ii): very little change in scoring occurred in Round 2.

Question 20: What is the probability that agricultural activities would prevent the establishment of an Eastern Sand Darter population across 10 years of reintroduction efforts in Big Otter Creek?

Context or clarifications provided: this question was similar to Question 17, but for Big Otter Creek as the recipient location. Additional context provided by a participant related to buffer zones being very sparse in the upper watershed, but more prevalent in the lower watershed. There are deep cut ravines on the Norfolk Sandplain (south of Otterville) because of clay runoff and erosion, so buffers have become more common. The southeast side of the watershed is similar. There are springs where the sand and clay meet under the till moraine, and erosion is common in these areas. Another participant inquired about water taking for agriculture, which occurs in the watershed and may be relevant. Approximately 1,000 water taking permits are issued in the watershed, and about 200 of those occur in Big Otter Creek. Adjacent wetland habitat that was used by ducks is no longer present, likely the result of water taking and nutrient issues from tobacco farming. Participants were asked to vote.

Results Round 1 (Figure 20.i): most points were allocated to the Low and Medium categories. A participant offered that they think agriculture is less intensive in Big Otter Creek than in the Thames or Grand rivers currently, and with shifts away from intensive tobacco farming in Big Otter Creek, they allocated more points to Medium and Low. Another participant allocated most of their points to Low and Very Low because of the positive changes in the watershed that mean agricultural practices are likely less of a concern now. A participant asked to see the results from Question 17 for the Ausable River, noting that more votes were allocated to lower categories for Big Otter Creek. Another participant noted that they skewed points higher than the rest of the group, stating that improvements don't necessarily mean conditions are good (i.e., better than bad doesn't necessarily mean good), and that there is still intensive row crop farming in the watershed that is likely comparable to the Ausable River. There are still drain enclosures and drain maintenance, and slicks of silt after heavy rain events. Another participant allocated most points to the lower categories based on the targeted habitat sampling that occurred in Big Otter Creek that suggested conditions were not so bad as to limit the potential effectiveness of an experimental reintroduction. The participant noted that relatively good aquatic habitat conditions still persisted despite stressors. Participants were asked to re-vote.

Results Round 2 (Figure 20.ii): very little change in the distribution of votes occurred in Round 2.

Question 21: What is the probability that Round Goby would prevent the establishment of an Eastern Sand Darter population across 10 years of reintroduction efforts in Big Otter Creek?

Context or clarifications provided: this question was similar to Question 18, but for Big Otter Creek as the recipient location. It was noted that Round Goby is known from throughout the historical range of Eastern Sand Darter in Big Otter Creek (i.e., where reintroduction is being considered). Recent work investigating impacts of Round Goby on other native darters in Big Otter Creek was discussed, noting that the density of Round Goby was highest in the downstream reaches near Lake Erie, but high-density patches were also known in the middle reaches. Another participant noted that Round Goby was associated with aquatic macrophytes in slow flowing areas of Big Otter Creek during recent sampling events. Participants were asked to vote.

Results Round 1 (Figure 21.i): most points were allocated to Medium, similar to the Ausable River. One participant shared that they allocated most points to Medium while spreading their points to the other categories. They considered that Channel Darter was able to mostly persist during Round Goby invasion, but since this question is about whether Eastern Sand Darter could establish (not just persist), it raised more concerns. Participants were asked to re-vote.

Results Round 2 (Figure 21.ii): there was very little change in Round 2 scoring.

Question 22: What is the probability that pathogens or parasites would prevent the establishment of an Eastern Sand Darter population across 10 years of reintroduction efforts in Big Otter Creek?

Context or clarifications provided: this question was similar to Question 19, but for Big Otter Creek as the recipient location. The issues around lack of data on diseases were briefly revisited. Participants were asked to vote.

Results Round 1 (Figure 22.i): most points were allocated to the Low category, as well as Medium and Very Low; the distribution of points was similar to the Ausable River. Participants were asked if they had any reason to believe the situation would be different in Big Otter Creek compared to the Ausable River; no differences were raised. Participants were asked to re-vote.

Results Round 2 (Figure 22.ii): there was no change in results from Round 1.

ECOLOGICAL RISKS

Question 23: What is the probability of an increased rate of inbreeding depression in the source population during, or three generations following, the removal of a) 250; b) 500; or, c) 1,000 Age-1+ individuals per year for up to 10 years?

Context or clarifications provided: the authors noted that there was limited information available to inform this section and asked for additional context or relevant examples from participants with genetics expertise. Definitions were reviewed: 1) inbreeding depression, which describes the relative reduction in fitness of offspring resulting from closely related individuals mating (rather than randomly mating); 2) outbreeding depression, which is a reduction in fitness from crossing of genetically distinct individuals, with fitness reduced below that of either parental population; 3) founder effect, which results from founding a new population from individuals that are not representative of the species genetic pool. The original wording of the question stated "increased rate of inbreeding in the source population", and a participant suggested a change to "increased rate of inbreeding depression in the source population" to imply that there was harm associated. Another participant agreed with this suggestion, noting that inbreeding itself does not necessarily lead to depression. Where inbreeding depression occurs, there is an increased genetic load from mating between closely related individuals. Some aspects of outbreeding can be positive (i.e., bringing in new genetic resources for selection to act on), but outbreeding depression that results in chromosomal rearrangements can be severe. Nevertheless, this is unlikely here given the shared post-glacial history. Additional explanation of the results presented from recent genetic work was provided by a participant, and they felt the evidence for inbreeding may not be as strong as was suggested. The authors noted that they did not distinguish between source populations for this question because although the population sizes likely differ between the Grand and Thames rivers, the proposed removal sizes were small relative to the estimated population sizes. Another participant agreed that the proposed removal sizes were unlikely to have a negative effect on population genetic diversity. They would be concerned if 1,000 individuals were removed from the same site every year for 10 years, but it was reiterated that removals would occur across the distribution in either source river (aligning with the assumptions of the population model). In response to a participant question, it was confirmed that Eastern Sand Darter is a diploid fish. Participants were asked to vote.

Results Round 1 (Figure 23a.i, 23b.i, 23c.i): most points were allocated to the Very Low category; there was a slight shift towards the higher categories as it progressed from part a) through c), but Very Low still dominated. One participant stated that they placed all points in the Very Low category because removal effort would be allocated randomly across the source population. Another participant referred to this question and responses as a ‘slam-dunk’, meaning that there appeared to be a very low chance that inbreeding depression in the source population would occur. Participants were asked to re-vote.

Results Round 2 (Figure 23a.ii, 23b.ii, 23c.ii): There was a slight shift of even more points towards Very Low, but generally little change in results from Round 1.

Question 24: What is the probability of inbreeding depression in the reintroduced population during, or three generations following, the addition of a) 250; b) 500; or, c) 1,000 Age-1+ individuals per year for up to 10 years?

Context or clarifications provided: a participant noted that their response would be very different one year after a reintroduction compared to year 9 or 10, and the authors clarified that this should be at the end of the reintroduction period (i.e., given that 10 years of reintroductions have occurred). Another participant echoed that reintroductions in this case are not a one-time event, and the level of inbreeding will increase inversely with twice the population size. They noted that there is a small increase in inbreeding from the first founding event (but not inbreeding depression). Even if starting with 250 individuals and 80% mortality, 50 surviving individuals would have a 1% inbreeding rate, which is a very low risk. A participant inquired about the risk of inbreeding depression given that there are no individuals present at the introduction site (i.e., first introduction event). There would be no inbreeding depression in the founding individuals, but it would appear in their offspring and grand-offspring. It was further explained that a founding effect can result not only in inbreeding depression, but also in a change of phenotypic expression from epistatic release where the genetic control traits are altered; however, a much smaller founding population would be needed for this to occur. Another participant inquired about how small the modelled reintroduced populations became throughout the 10-year reintroduction phase during the high mortality scenarios. They felt that the risk of inbreeding depression depended on how small the reintroduced populations were, and what kind of bottleneck they experienced; high mortality coupled with random stochasticity resulting in very low abundances could be risky. Another participant agreed that this could be risky if the reintroduction was a single event, but given that new individuals would be added each year, the overall risk of inbreeding depression was low (noting again that inbreeding may occur, but will not necessarily result in inbreeding depression). Aurora Trout (*Salvelinus fontinalis timagamiensis*) was raised as an example, where a founding population of six individuals resulted in a population that has sustained itself for 70 years. Participants were asked to vote.

Results Round 1 (Figure 24a.i, 24b.i, 24c.i): overwhelmingly Very Low with limited spread to other categories. Participants were asked to re-vote.

Results Round 2 (Figure 24a.ii, 24b.ii, 24c.ii): no changes.

Question 25: What is the probability of a founder effect occurring in the recipient location given the reintroduction of a) 250; b) 500; or, c) 1,000 Age-1+ individuals per year for up to 10 years?

Context or clarifications provided: a participant offered that a reintroduction could result in an unintentionally biased subsample of the population that does not capture the representative genetic diversity, which can have consequences for the founding population. This would likely be of greater concern if only one reintroduction event were to occur, but given that there would be multiple reintroduction events from independent, random sampling from the source populations it is unlikely. Participants were asked to vote.

Results Round 1 (Figure 25a.i, 25b.i, 25c.i): the points were allocated mostly to the Very Low category again for all three parts (a, b, and c), with a slight shift towards Very Low as the number of introduced individuals increased (i.e., when moving from part a) through c)). One participant felt unsure about the genetic consequences, so they allocated 20 points to each category. Participants were asked to re-vote.

Results Round 1 (Figure 25a.ii, 25b.ii, 25c.ii): no changes from Round 1.

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?

Context or clarifications provided: the authors reviewed information from a recent genetics study used to inform this question, noting that the Grand and Thames rivers showed evidence of distinctiveness based on neutral genetic markers. The possibility of heterotic effects or mixing to produce the most robust Eastern Sand Darter was discussed, but ultimately was unknown. Occasionally, a boost in fitness in the first generation is observed (heterosis), but any issues resulting from outbreeding depression are unlikely to appear until later generations. The possibility of genetic rescue was raised, where new genetic material to a small population can provide a benefit. A participant flagged an additional study that may be able to contribute to this topic related to hybridization of two closely related darter species. Participants were asked to vote.

Results Round 1 (Figure 26.i): points were fairly spread out, but the most points were in the Low, Medium and Very Low categories. Six people identified complete uncertainty to this question, allocating 20 points to each category. A participant explained that, typically, outbreeding depression is a concern for hybrids of closely related species and is less of a concern for populations of a single species with a shared post-glacial history like in this case, unless the habitat conditions (and selection pressures) are drastically different. A participant asked if the higher turbidity in the Thames River compared to the Grand River, which likely began approximately 100 years ago, could be a significant enough stressor to have an effect. It was explained that this would depend on the population size, abundance of a trait in that population, and the strength of selection acting on the trait. For a trait in a small population under strong selection, it could take approximately 20 years to become fixed, but would take longer in a large population as it needs to spread more. Participants were asked to re-vote.

Results Round 2 (Figure 26.ii): results shifted slightly towards Low and Very Low.

Question 27: What is the probability of interspecific hybridization in the recipient location?

Context or clarifications provided: the authors explained that interspecific hybridization would go against the fundamental objective of a new population of the species. A participant raised that natural hybridization between several darter species has been documented, as well as some intergeneric hybridization between *Percina* spp. and *Etheostoma* spp., but none has been reported with *Ammocrypta* spp. Participants were asked to vote.

Results Round 1 (Figure 27.i): most points were allocated to Very Low with very little spread in other categories. Participants were asked to revote.

Results Round 2 (Figure 27.ii): there was a very subtle shift toward Very Low.

Question 28: a) What is the probability that reintroduction would introduce novel pathogens or parasites to the recipient location? b) What is the probability that introduced novel pathogens or parasites would cause significant harm to the recipient freshwater community?

Context or clarifications provided: the authors reiterated from earlier discussions that there was great uncertainty on the topic of diseases in freshwater ecosystems in general. They added that field crews take measures to reduce spreading organisms between watersheds. A proposed rationale for answering this question was whether participants believed the disease communities are similar between potential source and recipient locations. This question originally did not include the “novel” descriptor, but was revised to include it.

Results Round 1 (Figure 28a.i, 28b.i): most points were allocated to the Low and Very Low categories for part a), but a few participants spread points evenly across categories. One participant offered that they allocated most points to Medium, and spread a few to High and Low as well. Both the Grand and Thames river populations are relatively large, and it’s possible that could result in a heavier disease load compared to fishes in the Ausable River or Big Otter Creek. Another participant stated they were debating whether or not a novel pathogen could have a population-level effect that we would not be aware of, and if not then we have no information, so they allocated points evenly across all categories. Another participant felt that the risk of disease transfer was likely higher from highly mobile species that are moving between watersheds already than from the proposed reintroduction events; most of the biggest disease outbreaks have come from outside of the Great Lakes and from non-native fishes. For part b), most points were still allocated to Low and Very low, but with more spread into higher categories. One participant noted that part a) is the probability of introduction of a novel disease, and part b) is the probability of a high impact given that introduction, and they felt that even a single disease introduction has the potential to have an extremely high impact, especially if it is novel and there is no resistance, so they allocated more points towards the Very High and High probability categories for part b) compared to part a). Participants were asked to re-vote.

Results Round 2 (Figure 28a.ii, 28b.ii): there was little change for part a), but points spread out more for part b) with many points moving from Very Low towards Medium.

Question 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?

Context or clarifications provided: the authors explained that their rationale for this question was that species removals or additions to ecosystems can cause drastic ecological changes (e.g., species removals in some circumstances can lead to food web alterations following harvesting pressure; species additions in some circumstances can lead to food web alterations, such as during species invasions). They noted this question was intentionally broad. Participants were asked to vote.

Results Round 1 (Figure 29.i): points were mostly allocated to the Low and Very Low probability categories. A participant noted that they were surprised to see so many votes in the Medium to Very High categories and wondered how others had interpreted “transformative changes”, as they were thinking of fundamental restructuring as a result of removals. Given that removals would occur across the entire distribution, they allocated almost all points to Very Low. Another participant responded that they felt it depended on how the removals took place and whether individuals were harvested from the same spot each time or spread out; it was reiterated that removals would occur across the distribution in the source river. Another participant responded that they didn't allocate any points in High or Very High, but did add a few to Medium, citing the removal of 1,000 individuals from Questions 2 and 4 was where the group started to get uncomfortable about the possibility of impacts to the source population. If the source population was harmed or lost, then there would be wider effects to the fish community. Another participant agreed with the first, that they were surprised to see points in the higher probability categories. Although the group did identify potential risks to the source population associated with removals of 1,000 individuals, those weren't risks around losing the species entirely, but were risks around reducing the abundance relative to the MVP thresholds. In terms of numbers in the whole fish community, 1,000 individuals is a relatively small number, and if transformative changes were expected to be even moderately probable, we would see those changes occurring from activities like light fishing pressure (which likely removes more individuals each year). Participants were asked to re-vote.

Results Round 2 (Figure 29.ii): points shifted towards Very Low and Low from Medium.

Question 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?

Context or clarifications provided: this question was similar to Question 29, but for the recipient locations. An author clarified that the establishment of Eastern Sand Darter in the recipient location was not to be considered as a transformative change as that is the goal of the reintroduction effort, but rather this question seeks to understand unintended consequences to other parts of the ecosystem. Participants were asked to vote.

Results Round 1 (Figure 30.i): most points were allocated to the Very Low and Low probability categories. A participant noted that they were not surprised by this result – they felt that the probability of changes to the ecosystem would be more likely from additions to the recipient location (as additions of 1,000 would be expected to turn into many more over time resulting from reproduction), compared to the removals from the source population (i.e., Question 29), but still a low overall chance of ecosystem restructuring. Another participant added that they also felt the probability of transformative changes would be low because the species either brought something in with it that caused harm (which was covered in earlier questions related to parasites/pathogens/diseases), or else the reintroduction was successful and likely the species has slowly established and not much shifts. Participants were asked to re-vote.

Results Round 2 (Figure 30.ii): a slight shift towards Very Low occurred.

SUMMARY OF MODIFIED DELPHI RESULTS

The authors presented a set of summary figures of the second round of voting, with one set related to ecological feasibility and the other to ecological risks. The results were scaled to one, the Very High and High probability categories were combined, and the range of values were presented with low, medium, and high, represented by green, yellow, and red colours, respectively. Effectively, the proportion of votes that were attributed to the High and Very High bins were presented to provide an overall likelihood of success. If the error bar was short, it indicated that most points were attributed to those categories. An annotation indicating the number of participants who attributed 20 points to each category could be added to each question. It was flagged that using green, yellow, and red might imply "go ahead" or "stop" with the reintroduction, which could be misinterpreted as a judgement or direction on implementation from the group. Another set of figures was presented that represented probability distributions, where the probability categories were resampled and a mean probability that the question was true could be derived. Some participants felt this presentation was more intuitive, but preferred the look of the summary figures. The individual plots from each question were also shared with the group as a package to help guide summary bullet development. Other options for presenting the results of the Delphi questions were discussed. Violin plots (instead of means) were a popular option as they could combine the value of the resampling approach in showing the distribution of probabilities with the visual appeal of the summary figures; however, the bins were categorical, making a violin plot challenging. A re-labeling of the x-axis on the dashboard figure was also proposed to improve clarity of what was being presented. The possibility of lumping the Medium category points in with High and Very High in the dashboard figure was discussed, but ultimately this would represent more than 50% of the probability, so it would be less informative than a coin flip as to whether that factor was important. Presenting geometric means was discussed as an option, but if there was a probability category that received no points, then the geometric mean of the question would be zero. Looking at whether groups of participants with similar/shared expertise responded similarly was another proposed idea for thinking about the group results. The authors continued to adjust the figures to try to incorporate the suggestions from the group, and it was agreed that both sets of figures could be presented in the Science Advisory Report (SAR), and participants could decide upon seeing the full document which figure was most informative, or if both were valuable.

Considerations and Feedback of the Modified Delphi process

As the meeting progressed, it became obvious where knowledge and data gaps existed to inform reintroduction efforts (e.g., considerations of biotic factors were a major gap). There was a reduction in definitive information the further into the questions the group progressed, and this was felt to be an important outcome to show where we had evidence and where we were lacking information and how that may affect decision-making. While complete certainty on reintroduction topics was unlikely to occur, it was hoped that managers can look at uncertainties that the group felt were the most important for making the decision, and weigh that against their own objectives.

Several participants raised concerns around the probability categories being unevenly distributed (i.e., Very High being > 95% but High being 67–95%), and this affected how they scored. They were cautious about placing many points in the Very High and Very Low categories, given how certain they felt they needed to be. One of these participants flagged their surprise to see so many votes allocated to the Very High category during Question 1 (for example), given all the uncertainties and assumptions in the population model. This was again raised at the end of the meeting when the summary figures were presented that summed the results from the Very High and High categories, the concern being that these were not representing the same proportions yet were weighted the same in the end. The value of having the probability categories being worth different values was lost when they were lumped together. Participants advocated for the roll-up method and/or proposed summaries to be presented at the beginning of the meeting so participants could better understand how their expert opinions were to be captured. Creating probability categories of equal value was raised as a potential easier path forward.

A participant noted (during Question 16) that some of the questions were asked once for the Ausable River and once for Big Otter Creek, but in many cases, information was equally limited, so participants were applying the same scores with the same rationales. This felt like re-voting on the same question four times.

A participant raised that the probabilities may have represented different things to different people. Some may have interpreted the questions as “if this happened 1,000 times, how many of those events would meet the criteria”, which more closely aligns with a true probability.

Others may have looked at what seems more or less probable or plausible, which fits better with the probability categories used. The authors asked if this participant would prefer a continuous approach as opposed to the categorical approach used here. This point was intended as more “food for thought” for future applications.

Overall, participants liked the modified Delphi approach. They felt the structured approach was a much more effective and quantitative way of gathering expert opinion than traditional peer-review meetings. It was intimidating at first, and occasionally questions felt rushed, but the flow improved as the group progressed through the questions and became more familiar with the approach. The group felt more engaged than in a traditional meeting as they were required to actively participate in each question, but also noted that it is more work for participants as they need to be extremely familiar with the content of the working paper. Careful consideration of the phrasing of the questions, good organization, and having the right expertise in the room were considered the most important drivers for a successful Delphi approach. Continued thought into how results should be summarized and presented for future iterations was recommended.

DRAFTING OF SUMMARY BULLETS AND SCIENCE ADVISORY REPORT

Draft SAR summary bullets were presented by the author team. It was stated that consensus was needed on the bullets before the meeting could conclude and participants were encouraged to contribute to finalizing the content and specific wording of the bullets. The authors provided an overview of the bullets, noting the first few pertained to the objectives, the next set were derived from the results of the working paper, and the final few were intended to the summarize the results of the expert judgement from the modified Delphi approach. Major discussions related to: linking the objectives of the peer-review meeting to the objectives in the species' Recovery Strategy, but the group felt this was best left for the body of the SAR; the level of detail to be included with the modelling results, as some parameters may be important for interpreting values presented (e.g., population sizes) but might also be too in-depth for the bullets; threats being considered in isolation and cumulative effects were not evaluated; whether or not “wild” was implied as the type of translocation and whether it was important to acknowledge that captive rearing for translocations was not evaluated here, given that it is mentioned in the Recovery Strategy; and, how best to capture group uncertainty, where phrasing of “more divergence among individuals for some factors and less variability among individuals for other factors” was thought to balance which topics had the highest uncertainty without giving the false impression that nothing was known and a decision could not be made. Minor edits also related to: consistency in how the source rivers were referred to (i.e., populations, rivers, watersheds, and ecosystems had all been used); including a depth threshold instead of the term “non-wadable”, as a depth threshold could be more meaningful for Eastern Sand Darter; wording changes to highlight that disease couldn't be thoroughly assessed because of a lack of empirical data; use of the word “risk” and whether “unintended (ecological) consequences” or “unintended harm” was more specific while still being a plain language term.

The Sources of Uncertainty were also discussed by the group. The author team again presented a bulleted list, and the group was encouraged to think about the discussions over the course of the meeting and identify any items that were not captured in the list. A few additions and/or modifications included: spatial distribution of habitats, population spatial structure, availability of suitable habitat (specifically related to the resolution of fines from the sidescan work), increased specificity of life-history traits that were uncertain, and population trajectory. Lastly, although it was not discussed during the meeting, a participant raised the issue of climate change being a large unknown that could limit success.

Lastly, the group felt that an additional consideration for interpreting the advice was that removals would be spread out throughout the source rivers and would not come from a small number of ‘hot-spots’. This idea was raised multiple times over the course of the meeting pertaining to many questions. It was an important assumption in the population models and should be considered when evaluating the technical feasibility of undertaking removals for reintroduction.

NEXT STEPS

The authors agreed to make the minor revisions to the working paper identified during the group review, and participants agreed it could be finalized as a Research Document. It did not need to be reviewed by participants again. The Science Advisory Report (SAR) would be finalized and include the revised summary figures. The SAR and Proceedings would be circulated to participants for a final review.

REFERENCES

Dextrase, A.J. 2013. Modelling occupancy and abundance of Eastern Sand Darter (*Ammocrypta pellucida*) while accounting for imperfect detection. Thesis (PhD) Trent University, Peterborough, ON, 352 p.

Firth, B.L., Poesch, M.S., Koops, M.A., Drake, D.A.R., and Power, M. 2021. Diet overlap of common and at-risk riverine benthic fishes before and after round goby (*Neogobius melanostomus*) invasion. Biol. Invasions 23: 221–234.

APPENDIX 1: TERMS OF REFERENCE

Evaluating the Ecological Benefits and Risks of an Experimental Eastern Sand Darter (*Ammocrypta pellucida*) Reintroduction in Ontario

Regional Science Peer Review – Ontario and Prairie Region

April 3-6, 2023
Virtual Meeting

Chairperson: Gilles Olivier

Context

Reintroduction is commonly identified in species recovery strategies or management plans as a potential method for improving the survival or recovery of species listed under the *Species at Risk Act* (SARA). This includes Eastern Sand Darter (*Ammocrypta pellucida*), a small-bodied freshwater fish species that has experienced extirpations across its Canadian range. The recovery strategy for Eastern Sand Darter (Ontario populations) identified reintroduction as a potential action that can help achieve population and distribution objectives (DFO 2012, Lamothe et al. 2021); however, reintroduction has yet to occur (Lamothe et al. 2019).

A national CSAS process recently provided guidance on how to evaluate the potential ecological benefits and risks of reintroduction to the survival or recovery of SARA-listed freshwater species and broader ecosystem components (Lamothe et al. 2022). For the current request, the recently developed national guidance document will be used to evaluate the potential change in survival or recovery of Eastern Sand Darter through an experimental reintroduction to a formerly occupied ecosystem in southwestern Ontario, including the uncertainty of factors that may influence the outcome of different reintroduction scenarios. Ultimately, this request will provide guidance for management regarding the use of reintroduction for Eastern Sand Darter recovery and will serve as a model for how to use the existing national guidance document to inform future decisions on the use of species reintroduction for improving the survival or recovery of other SARA-listed freshwater species.

Objectives

- Evaluate ecological factors that could influence successful reintroduction of Eastern Sand Darter in candidate recipient locations (Big Otter Creek; Ausable River) from candidate source populations (Grand River; Thames River);
- Evaluate the ecological risks associated with reintroduction of Eastern Sand Darter, both for the species and broader ecosystem at source and recipient locations; and
- Evaluate the change in survival or recovery of Eastern Sand Darter resulting from the re-establishment of a population in a formerly occupied location.

Expected Publications

- Science Advisory Report
- Proceedings
- Research Document

Expected Participation

- Fisheries and Oceans Canada (DFO)

- Ontario Ministry of Natural Resources and Forestry (MNRF)
- Academia
- Other invited experts

References

DFO. 2012. Recovery strategy for the Eastern Sand Darter (*Ammocrypta pellucida*) in Canada: Ontario populations. Species at Risk Act Recovery Strategy Series, Fisheries and Oceans Canada, Ottawa. vii + 58 pp.

Lamothe, K.A., Drake, D.A.R., Pitcher, T.E., Broome, J.E., Dextrase, A.J., Gillespie, A., Mandrak, N.E., Poesch, M.S., Reid, S.M., and Vachon, N. 2019. Reintroduction of fishes in Canada: a review of research progress for SARA-listed species. Environ. Rev. 27(4): 575–599.

Lamothe, K.A., van der Lee, A.S., Drake, D.A.R., and Koops, M.A. 2021. The translocation trade-off for eastern sand darter (*Ammocrypta pellucida*): balancing harm to source populations with the goal of re-establishment. Can. J. Fish. Aquat. Sci. 78(9): 1321–1331.

Lamothe, K.A., Morris, T.J., and Drake, D.A.R. 2022. Decision support framework for the conservation translocation of SARA-listed freshwater fishes and mussels. DFO Can. Sci. Advis. Sec. Res. Doc. 2022/064. vii + 83 p.

APPENDIX 2: MEETING AGENDA

Evaluating the Ecological Benefits and Risks of an Experimental Eastern Sand Darter (*Ammocrypta pellucida*) Reintroduction in Ontario

CSAS Regional Science Peer Review Meeting
Ontario and Prairie Region

April 3–6th, 2023
Virtual MS Teams

Chair: Gilles Olivier
Rapporteurs: Julia Colm, Lindsay Potts, Jennifer Diment

Agenda		
Day 1 – Monday April 3rd 10:00 am – 4:00 pm EST		
10:00–10:15	Introductions and Roundtable	Gilles Olivier
10:15–10:30	CSAS Peer Review Process	Joclyn Paulic (CSAS)
10:30–12:00	Presentation of working paper	Karl Lamothe
12:00–13:00	Lunch Break	-
13:00–16:00	Discussion of working paper (+15 min break as needed)	All
Day 2 – Tuesday April 4th 10:00 am – 4:00 pm EST		
10:00–10:15	Recap Day 1	Gilles Olivier/Karl Lamothe
10:15–12:00	Evaluating the probability of success: Delphi consensus-based approach	All
12:00–13:00	Lunch Break	-
13:00–16:00	Discussion/ questionnaire continued (+15 min break as needed)	All
Day 3 – Wednesday April 5th 10:00 am – 4:00 pm EST		
10:00–10:15	Recap Day 2	Gilles Olivier/Karl Lamothe
10:15–12:00	Evaluating the probability of unintended consequences: Delphi consensus-based approach	All
12:00–13:00	Lunch break	-
13:00–16:00	Discussion/ questionnaire continued (+15 min break as needed)	All
Day 4 – Thursday April 6th 10:00 am – 4:00 pm EST		
10:00–10:15	Recap Day 3	Gilles Olivier/Karl Lamothe
10:15–12:00	Draft Science Advisory Bullets	All
12:00–13:00	Lunch break	-
13:00–14:45	Draft Science Advisory Report	All
14:45–15:00	Final Remarks and Next Steps	Gilles Olivier

APPENDIX 3: LIST OF MEETING PARTICIPANTS

Name	Affiliation/Organization
Jason Barnucz	DFO – Science
Amy Boyko	DFO – Species at Risk Program
Jeremy Broome	DFO – Science
Julia Colm (Rapporteur)	DFO – Science
Al Dextrase	Ontario Ministry of Natural Resources and Forestry
Jennifer Diment (Rapporteur)	DFO – Science
Andrew Drake	DFO – Science
Britney Firth	University of Waterloo
Paul Gagnon	Long Point Region Conservation Authority
Bill Glass	DFO – Fish and Fish Habitat Protection Program
Paul Grant	DFO – Science
Kari Jean	Ausable Bayfield Conservation Authority
Marten Koops	DFO – Science
Karl Lamothe	DFO – Science
Nick Mandrak	University of Toronto Scarborough
Todd Morris	DFO – Science
Gilles Olivier (Chair)	DFO – Science
Joclyn Paulic	DFO – Canadian Science Advisory Secretariat
Trevor Pitcher	University of Windsor
Lindsay Potts (Rapporteur)	DFO – Science
Scott Reid	Ontario Ministry of Natural Resources and Forestry
Shawn Staton	DFO – Species at Risk Program
Wendylee Stott	DFO – Science
Adam van der Lee	DFO – Science
Chris Wilson	Ontario Ministry of Natural Resources and Forestry

APPENDIX 4: SUMMARY RESULTS OF GROUP CONSENSUS EXERCISE

Table A1. Scoring summary for questions asked of expert participants regarding the influence of factors on achieving a successful reintroduction outcome. Scores were aggregated across participants and scaled between 0 and 1. Questions are presented in Table 1 and Table 2.

Question	*Part*	*Very Low*	*Low*	*Medium*	*High*	*Very High*
1	a)	0.00	0.03	0.12	0.24	0.60
1	b)	0.01	0.06	0.22	0.26	0.46
1	c)	0.06	0.20	0.30	0.23	0.22
2	a)	0.03	0.05	0.17	0.30	0.45
2	b)	0.06	0.13	0.18	0.30	0.32
2	c)	0.15	0.18	0.29	0.22	0.16
3		0.35	0.46	0.15	0.03	0.01
4	a)	0.56	0.31	0.09	0.03	0.01
4	b)	0.53	0.32	0.10	0.03	0.02
4	c)	0.50	0.34	0.12	0.03	0.01
4	d)	0.48	0.35	0.12	0.03	0.02
5	a)	0.02	0.05	0.13	0.18	0.63
5	b)	0.04	0.13	0.23	0.31	0.29
5	c)	0.19	0.29	0.29	0.11	0.12
6	a)	0.04	0.15	0.22	0.30	0.28
6	b)	0.11	0.20	0.25	0.26	0.18
6	c)	0.23	0.36	0.23	0.08	0.09
7		0.34	0.42	0.21	0.03	0.00
8	a)	0.46	0.34	0.16	0.04	0.01
8	b)	0.44	0.34	0.16	0.05	0.02
8	c)	0.46	0.34	0.15	0.04	0.01
8	d)	0.44	0.33	0.17	0.05	0.02
9	a)	0.02	0.06	0.21	0.38	0.33
9	b)	0.05	0.15	0.46	0.25	0.08
9	c)	0.08	0.28	0.45	0.16	0.03
10		0.02	0.12	0.30	0.23	0.33
11	a)	0.01	0.06	0.08	0.35	0.50
11	b)	0.03	0.08	0.21	0.37	0.31
11	c)	0.04	0.15	0.29	0.34	0.18
12		0.00	0.01	0.11	0.40	0.48
13	a)	0.01	0.03	0.14	0.41	0.40
13	b)	0.04	0.12	0.31	0.38	0.15
13	c)	0.10	0.24	0.33	0.21	0.12
14	a)	0.02	0.07	0.10	0.36	0.46
14	b)	0.03	0.16	0.29	0.37	0.15
14	c)	0.13	0.22	0.29	0.24	0.11
15	a)	0.12	0.35	0.33	0.15	0.05
15	b)	0.12	0.34	0.35	0.14	0.05
16	a)	0.14	0.35	0.31	0.14	0.05
16	b)	0.14	0.35	0.33	0.14	0.05
17		0.07	0.26	0.38	0.20	0.09
18		0.09	0.28	0.43	0.17	0.04
19		0.19	0.36	0.25	0.12	0.08

Question	*Part*	*Very Low*	*Low*	*Medium*	*High*	*Very High*
20		0.09	0.39	0.32	0.15	0.05
21		0.04	0.19	0.45	0.23	0.08
22		0.21	0.38	0.21	0.12	0.08
23	a)	0.80	0.10	0.08	0.01	0.00
23	b)	0.79	0.11	0.08	0.01	0.00
23	c)	0.76	0.13	0.09	0.02	0.01
24	a)	0.79	0.14	0.05	0.01	0.01
24	b)	0.82	0.12	0.04	0.01	0.01
24	c)	0.85	0.10	0.03	0.01	0.01
25	a)	0.68	0.17	0.10	0.03	0.03
25	b)	0.72	0.15	0.08	0.03	0.02
25	c)	0.75	0.14	0.07	0.03	0.02
26		0.42	0.36	0.13	0.06	0.03
27		0.81	0.12	0.04	0.01	0.01
28	a)	0.42	0.33	0.12	0.08	0.05
28	b)	0.26	0.23	0.23	0.17	0.11
29		0.63	0.30	0.05	0.01	0.01
30		0.61	0.29	0.07	0.02	0.01

Table A2. Number of participants that identified complete uncertainty per question per round. Total number of participants per questions is also presented.

Question	*Part*	*Round 1 (count)*	*Round 1 (proportion)*	*Round 2 (count)*	*Round 2 (proportion)*	*Number of participants*
1	a)	1	0.059	0	-	17
1	b)	0	-	0	-	16
1	c)	0	-	0	-	16
2	a)	0	-	0	-	16
2	b)	0	-	0	-	16
2	c)	1	0.063	0	-	16
3	-	0	-	0	-	16
4	a)	0	-	0	-	16
4	b)	0	-	0	-	16
4	c)	0	-	0	-	16
4	d)	0	-	0	-	16
5	a)	0	-	0	-	15
5	b)	0	-	0	-	15
5	c)	0	-	0	-	16
6	a)	0	-	0	-	16
6	b)	0	-	0	-	16
6	c)	0	-	0	-	16
7	-	0	-	0	-	16
8	a)	0	-	0	-	16
8	b)	0	-	0	-	16
8	c)	0	-	0	-	16
8	d)	0	-	0	-	16
9	a)	0	-	0	-	16
9	b)	1	0.063	0	-	16
9	c)	1	0.063	0	-	16
10	-	1	0.063	0	-	16
11	a)	0	-	0	-	16
11	b)	0	-	0	-	16
11	c)	0	-	0	-	16
12	-	0	-	0	-	15
13	a)	0	-	0	-	15
13	b)	1	0.067	1	0.067	15
13	c)	3	0.200	3	0.200	15
14	a)	0	-	0	-	16
14	b)	0	-	0	-	16
14	c)	3	0.188	3	0.188	16
15	a)	0	-	0	-	17
15	b)	0	-	0	-	17
16	a)	0	-	0	-	17
16	b)	0	-	0	-	17
17	-	0	-	0	-	17
18	-	0	-	0	-	17
19	-	5	0.294	6	0.353	17
20	-	0	-	0	-	17
21	-	0	-	0	-	17

Question	*Part*	*Round 1 (count)*	*Round 1 (proportion)*	*Round 2 (count)*	*Round 2 (proportion)*	*Number of participants*
22	-	6	0.353	6	0.353	17
23	a)	0	-	0	-	17
23	b)	0	-	0	-	17
23	c)	0	-	0	-	17
24	a)	0	-	0	-	16
24	b)	0	-	0	-	16
24	c)	0	-	0	-	16
25	a)	1	0.063	1	0.063	16
25	b)	1	0.063	1	0.063	16
25	c)	1	0.063	1	0.063	16
26	-	6	0.400	2	0.133	15
27	-	1	0.067	1	0.067	15
28	a)	2	0.133	3	0.200	15
28	b)	4	0.267	2	0.133	15
29	-	0	-	0	-	16
30	-	0	-	0	-	16

Figures

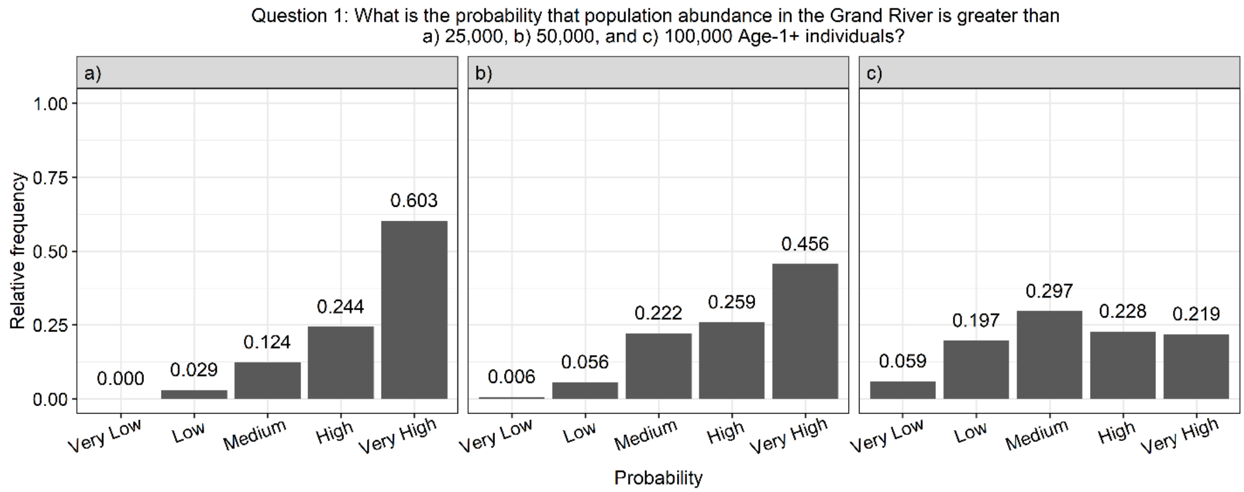

Figure A1. Aggregated participant responses to Question 1: What is the probability that population abundance in the Grand River is greater than a) 25,000, b) 50,000, and c) 100,000 age-1+ individuals? Responses scaled to 1.

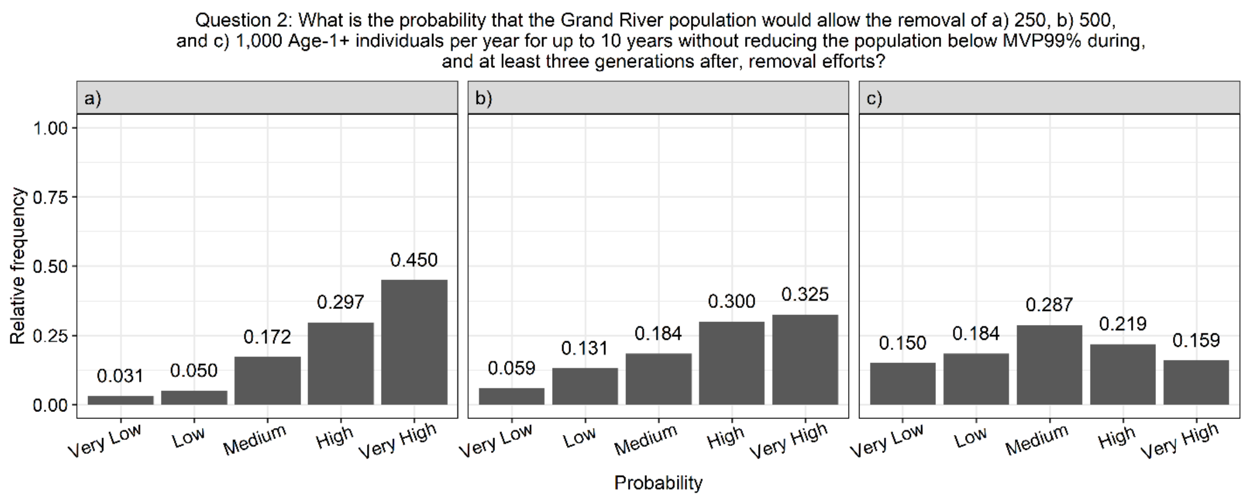

Figure A2. Aggregated participant responses to Question 2: What is the probability that the Grand River population would allow the removal of a) 250, b) 500, and c) 1,000 age-1+ individuals per year for up to 10 years without reducing the population below $MVP_{99\%}$ during, and at least three generations after, removal efforts? Responses scaled to 1.

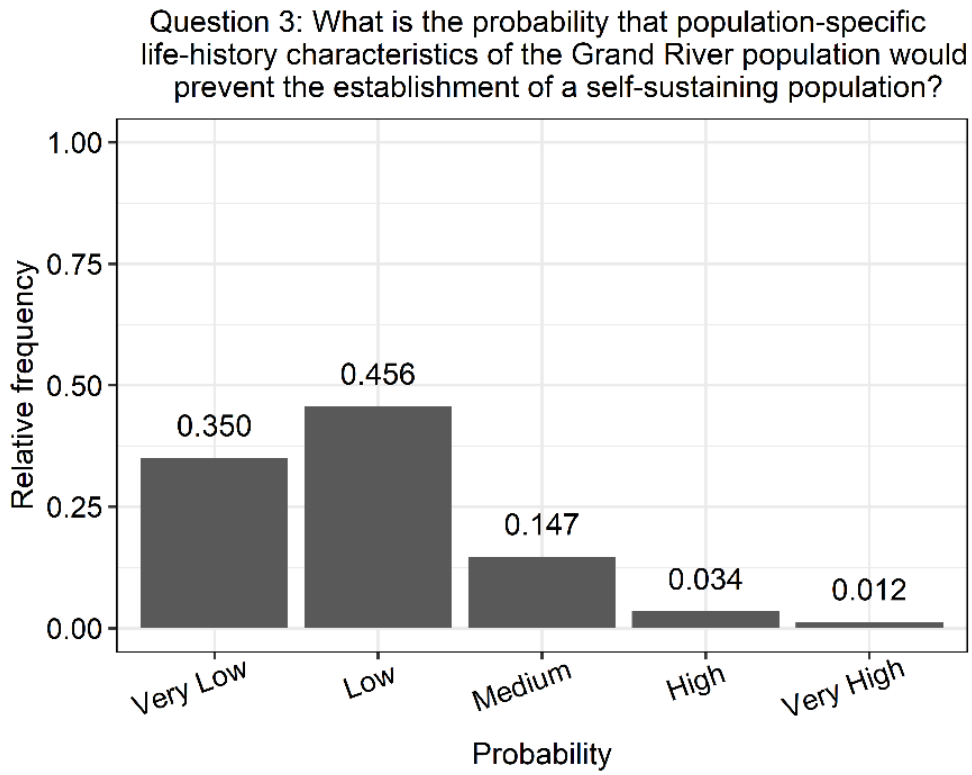

Figure A3. Aggregated participant responses to Question 3: What is the probability that population-specific life-history characteristics (e.g., age-at-maturity, fecundity, survival, growth, sex ratio) of the Grand River population would prevent the establishment of a self-sustaining population? Responses scaled to 1.

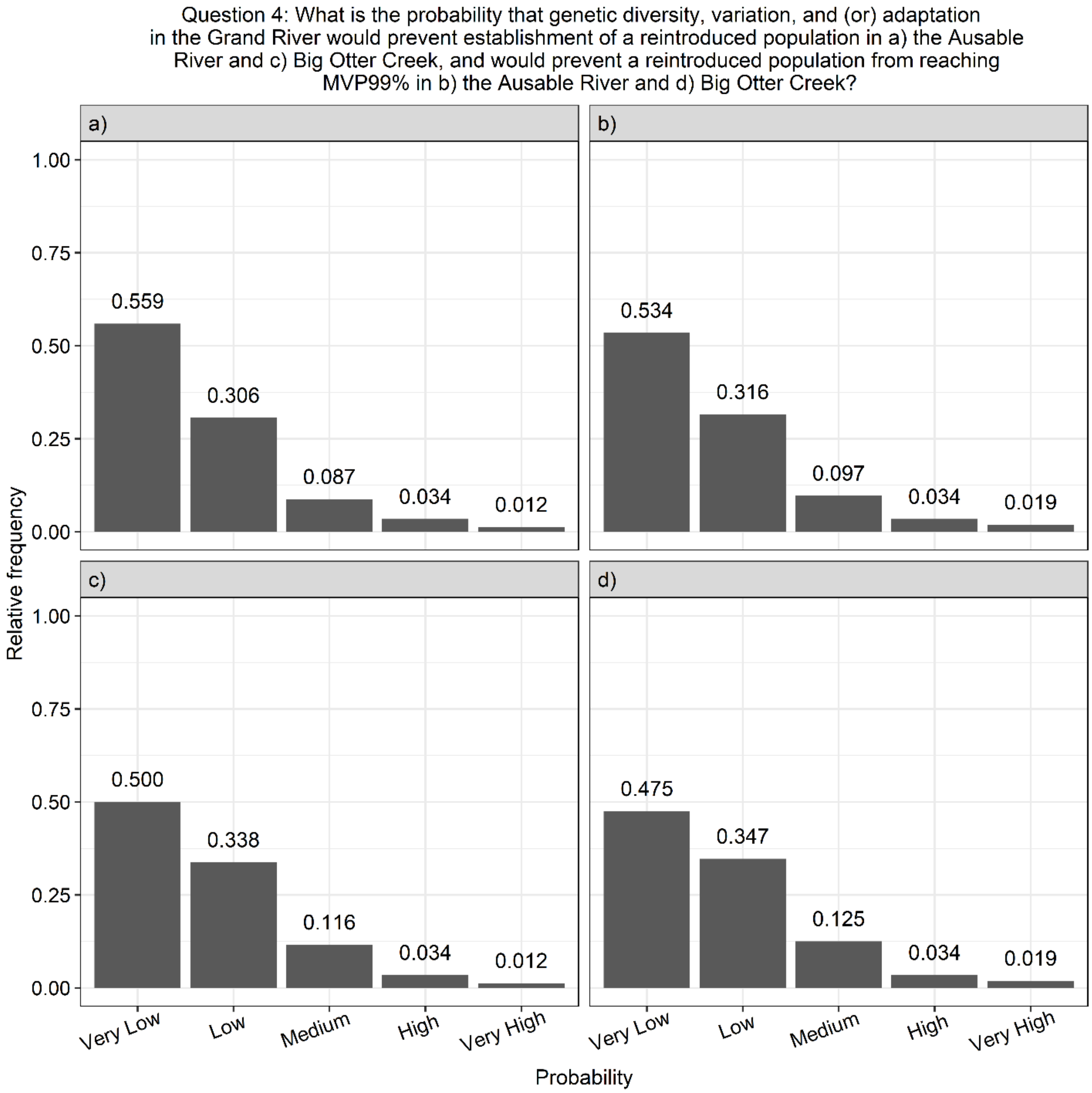

Figure A4. Aggregated participant responses to Question 4: What is the probability that genetic diversity, variation, and (or) adaptation in the Grand River would prevent establishment of a reintroduced population in a) the Ausable River and c) Big Otter Creek, and would prevent a reintroduced population from reaching $MVP_{99\%}$ in b) the Ausable River and d) Big Otter Creek? Responses scaled to 1.

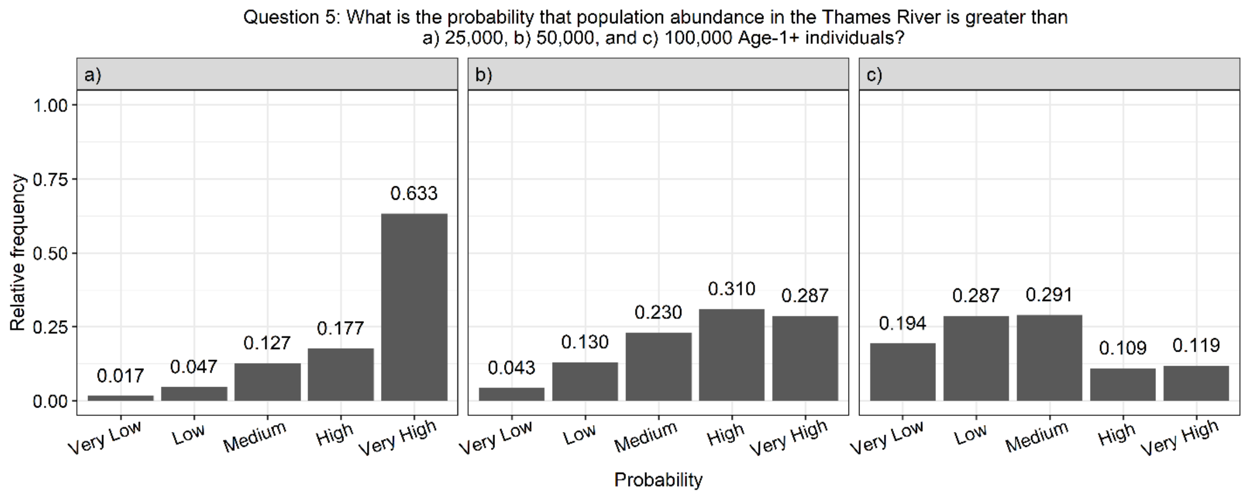

Figure A5. Aggregated participant responses to Question 5: What is the probability that population abundance in the Thames River is greater than a) 25,000, b) 50,000, and c) 100,000 age-1+ individuals? Responses scaled to 1.

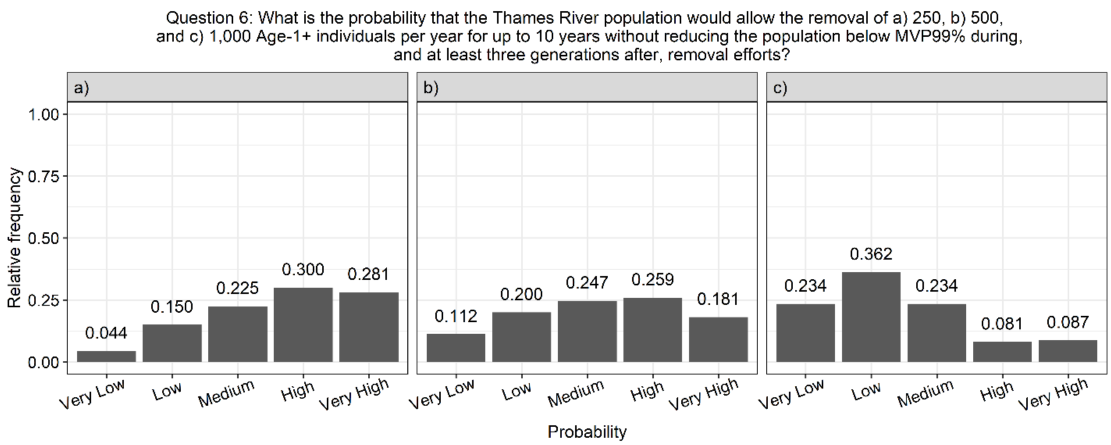

Figure A6. Aggregated participant responses to Question 6: What is the probability that the Thames River population would allow the removal of a) 250, b) 500, and c) 1,000 age-1+ individuals per year for up to 10 years without reducing the population below $MVP_{99\%}$ during, and at least three generations after, removal efforts? Responses scaled to 1.

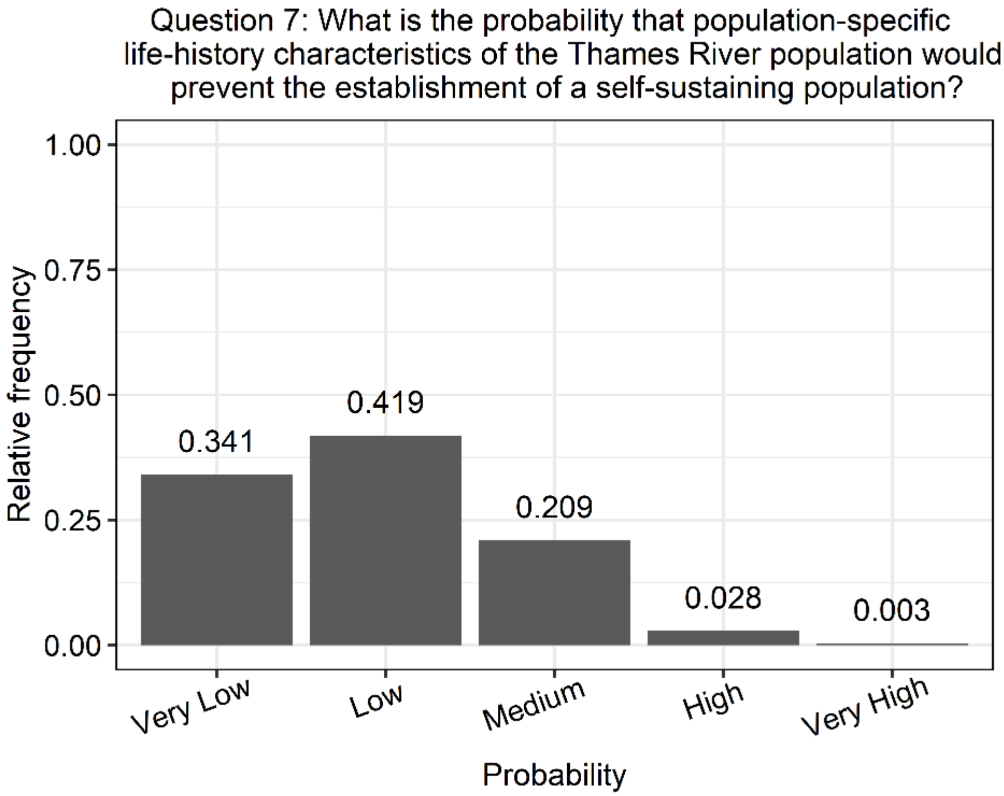

Figure A7. Aggregated participant responses to Question 7: What is the probability that population-specific life-history characteristics (e.g., age-at-maturity, fecundity, survival, growth, sex ratio) of the Thames River population would prevent the establishment of a self-sustaining population? Responses scaled to 1.

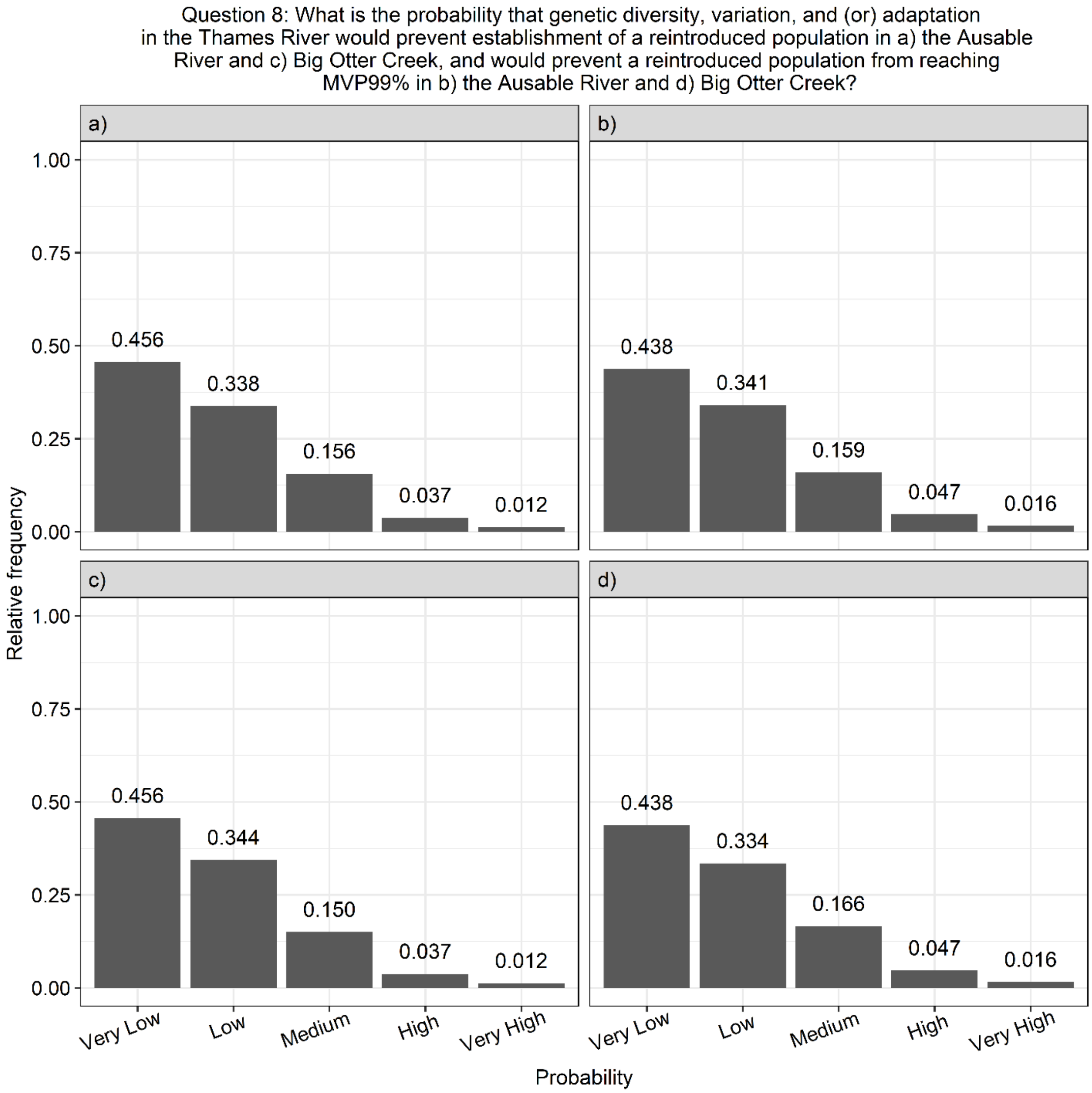

Figure A8. Aggregated participant responses to Question 8: What is the probability that genetic diversity, variation, and (or) adaptation in the Thames River would prevent establishment of a reintroduced population in a) the Ausable River and c) Big Otter Creek, and would prevent a reintroduced population from reaching $MVP_{99\%}$ in b) the Ausable River and d) Big Otter Creek? Responses scaled to 1.

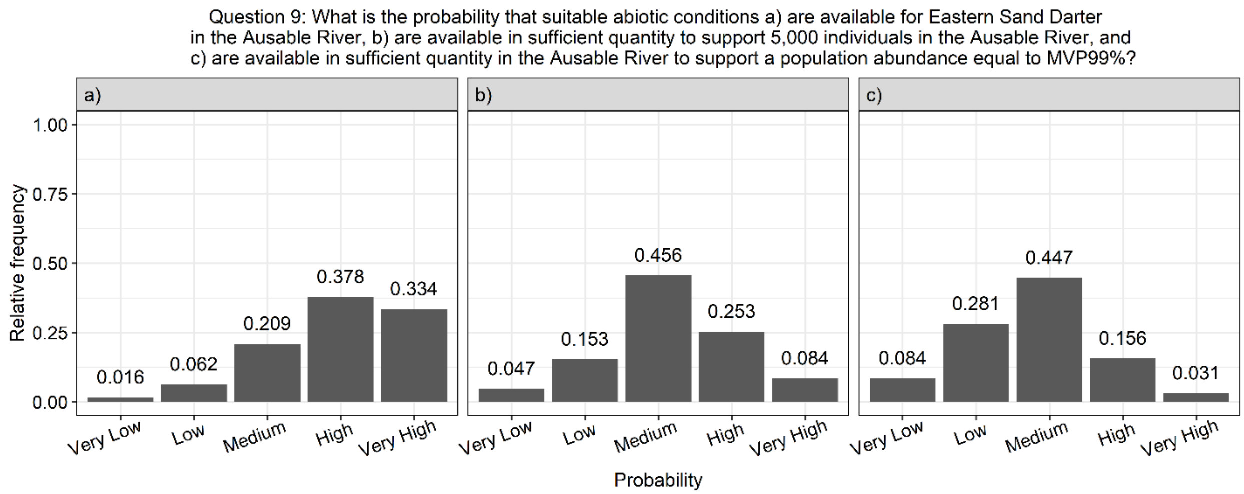

Figure A9. Aggregated participant responses to Question 9: What is the probability that suitable abiotic conditions are a) available for Eastern Sand Darter in the Ausable River, b) available in sufficient quantity to support 5,000 individuals in the Ausable River, and c) available in sufficient quantity in the Ausable River to support a population abundance equal to $MVP_{99\%}$*? Responses scaled to 1.*

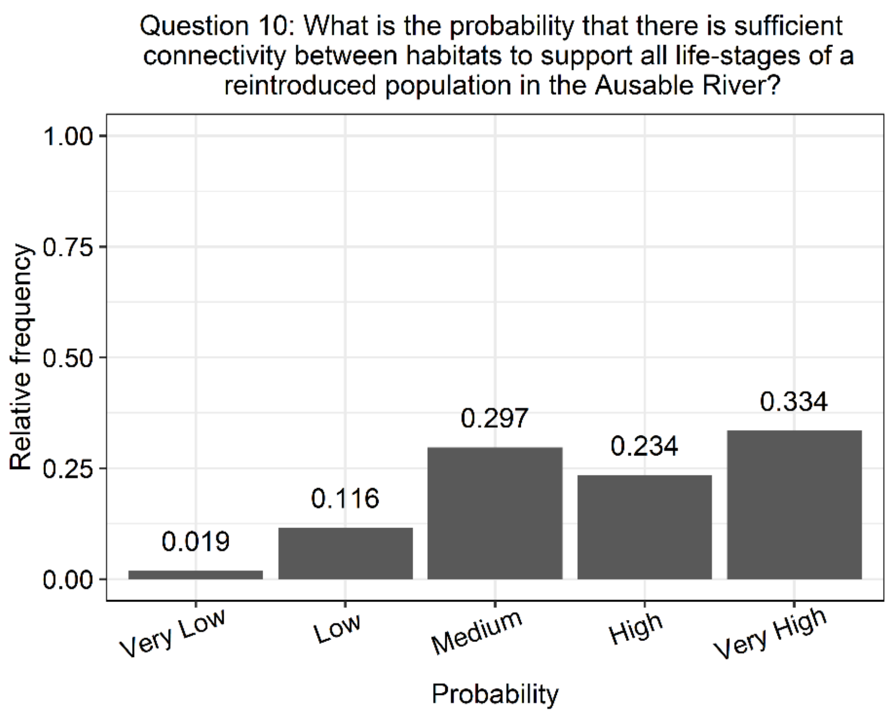

Figure A10. Aggregated participant responses to Question 10: What is the probability that there is sufficient connectivity between habitats to support all life-stages of a reintroduced population in the Ausable River? Responses scaled to 1.

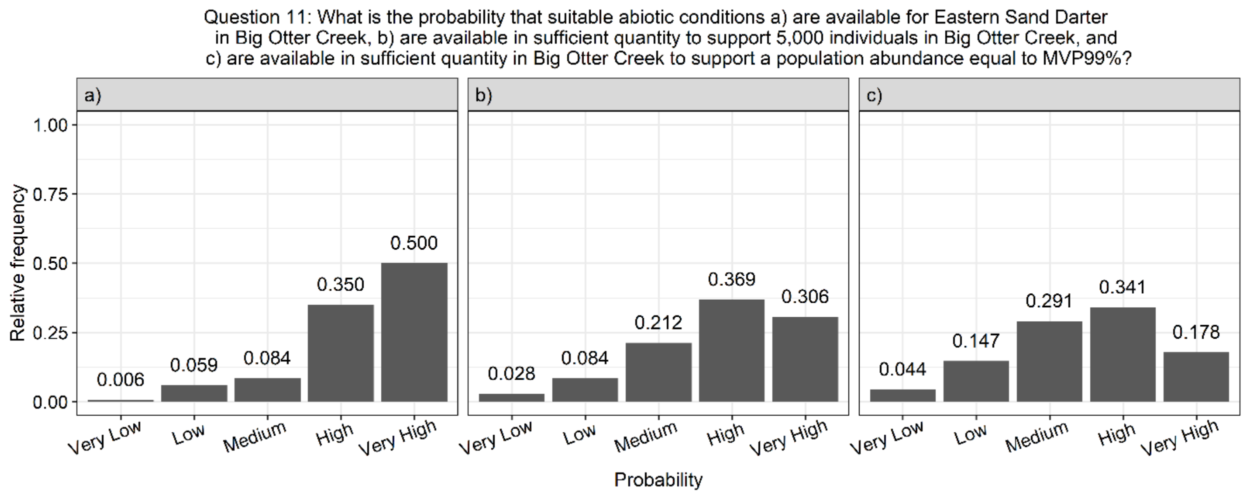

Figure A11. Aggregated participant responses to Question 11: What is the probability that suitable abiotic conditions are a) available for Eastern Sand Darter in Big Otter Creek, b) available in sufficient quantity to support 5,000 individuals in Big Otter Creek, and c) available in sufficient quantity in Big Otter Creek to support a population abundance equal to $MVP_{99\%}$*? Responses scaled to 1.*

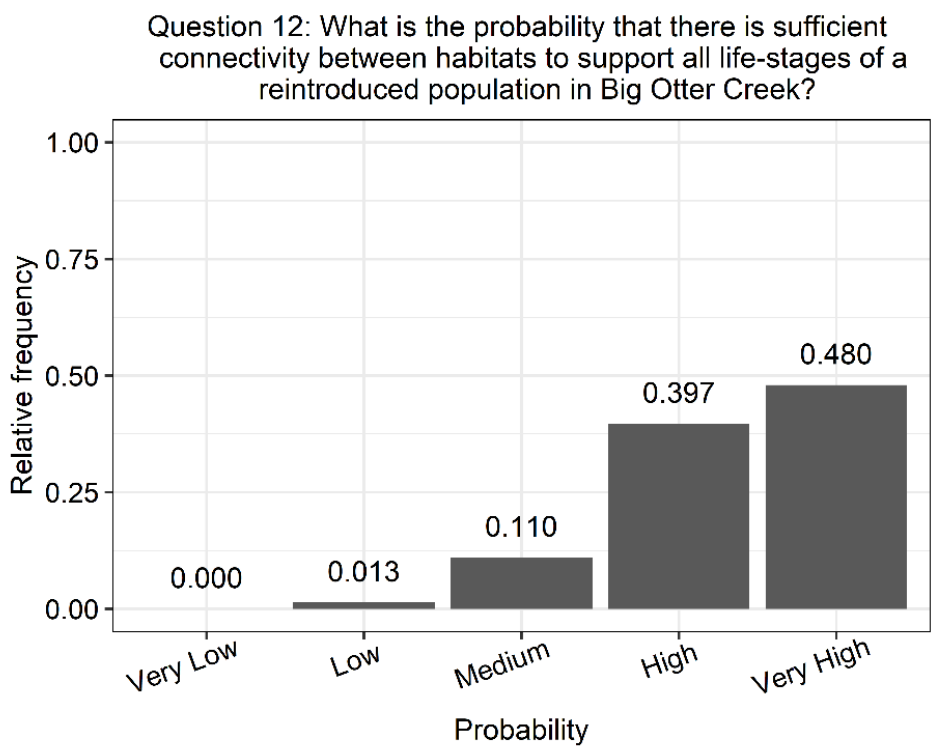

Figure A12. Aggregated participant responses to Question 12: What is the probability that there is sufficient connectivity between habitats to support all life-stages of a reintroduced population in Big Otter Creek? Responses scaled to 1.

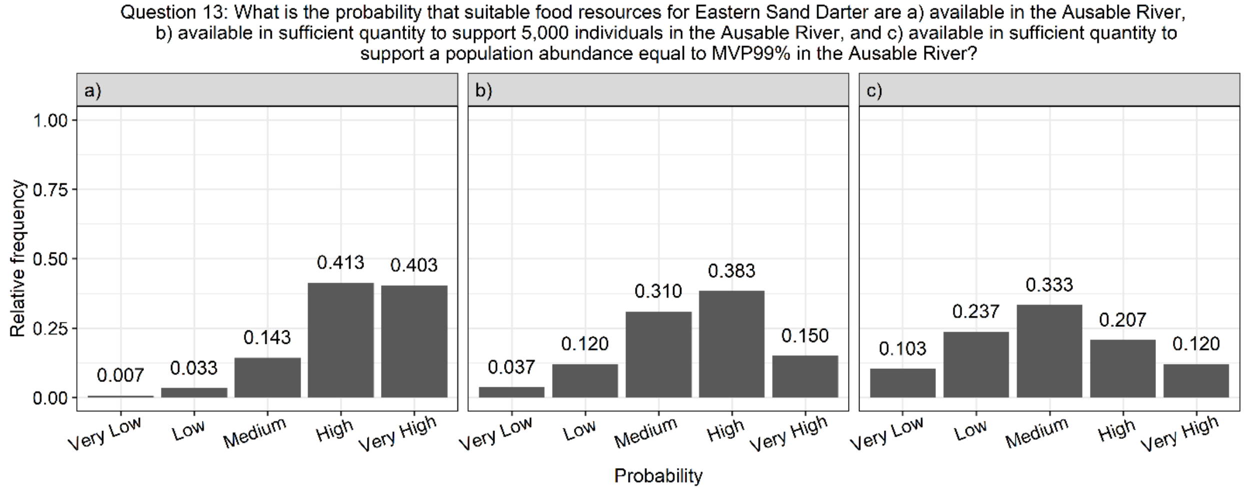

Figure A13. Aggregated participant responses to Question 13: What is the probability that suitable food resources are a) available for Eastern Sand Darter in the Ausable River, b) available in sufficient quantity to support 5,000 individuals in the Ausable River, and c) available in sufficient quantity in the Ausable River to support a population abundance equal to $MVP_{99\%}$*? Responses scaled to 1.*

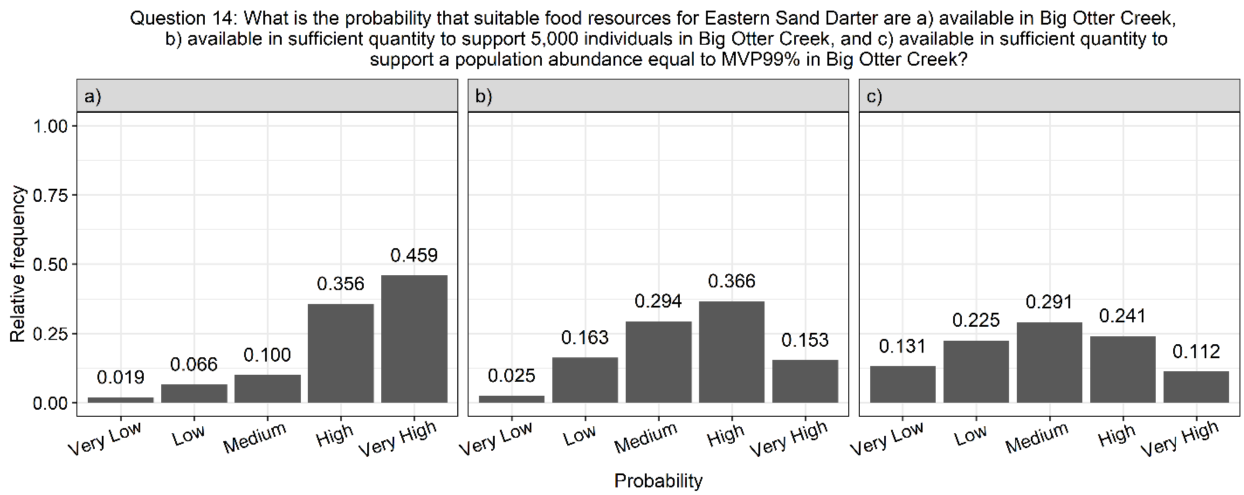

Figure A14. Aggregated participant responses to Question 14: What is the probability that suitable food resources are a) available for Eastern Sand Darter in Big Otter Creek, b) available in sufficient quantity to support 5,000 individuals in Big Otter Creek, and c) available in sufficient quantity in Big Otter Creek to support a population abundance equal to $MVP_{99\%}$*? Responses scaled to 1.*

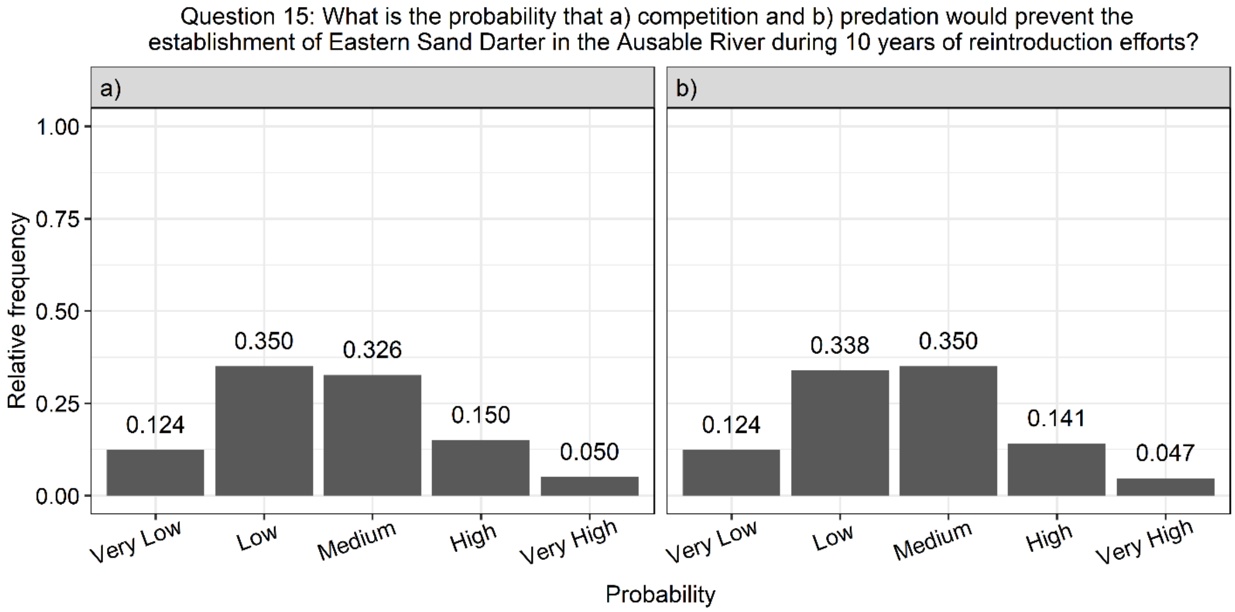

Figure A15. Aggregated participant responses to Question 15: What is the probability that a) competition and b) predation would prevent the establishment of Eastern Sand Darter in the Ausable River during 10 years of reintroduction efforts? Responses scaled to 1.

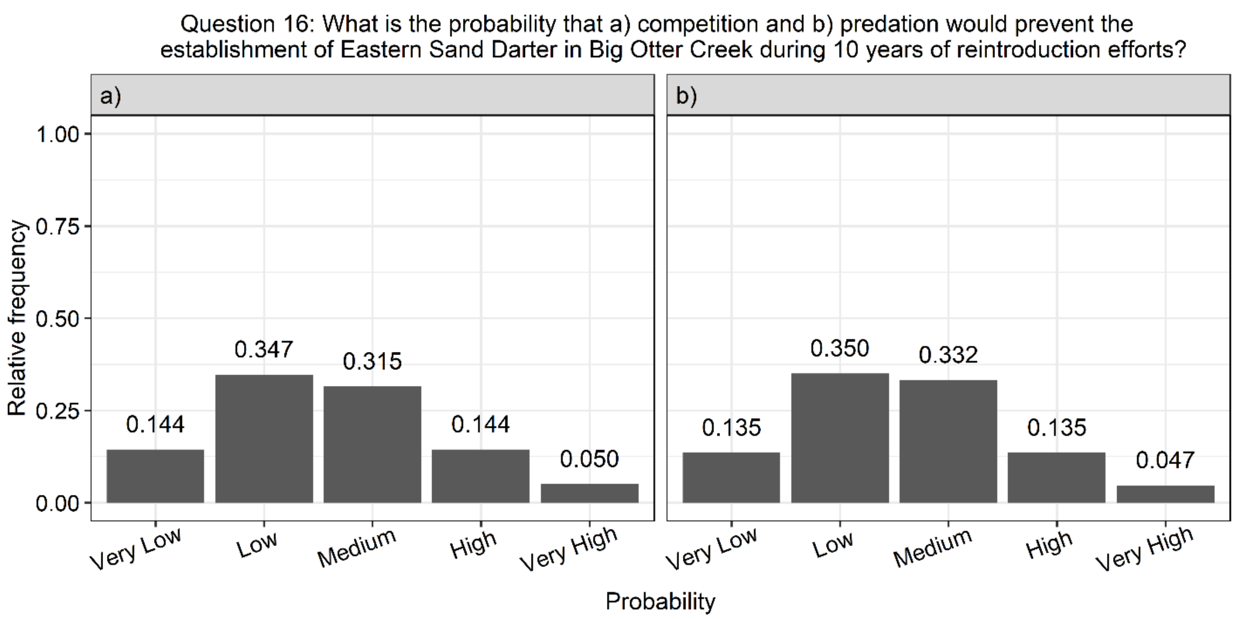

Figure A16. Aggregated participant responses to Question 16: What is the probability that a) competition and b) predation would prevent the establishment of Eastern Sand Darter in Big Otter Creek during 10 years of reintroduction efforts? Responses scaled to 1.

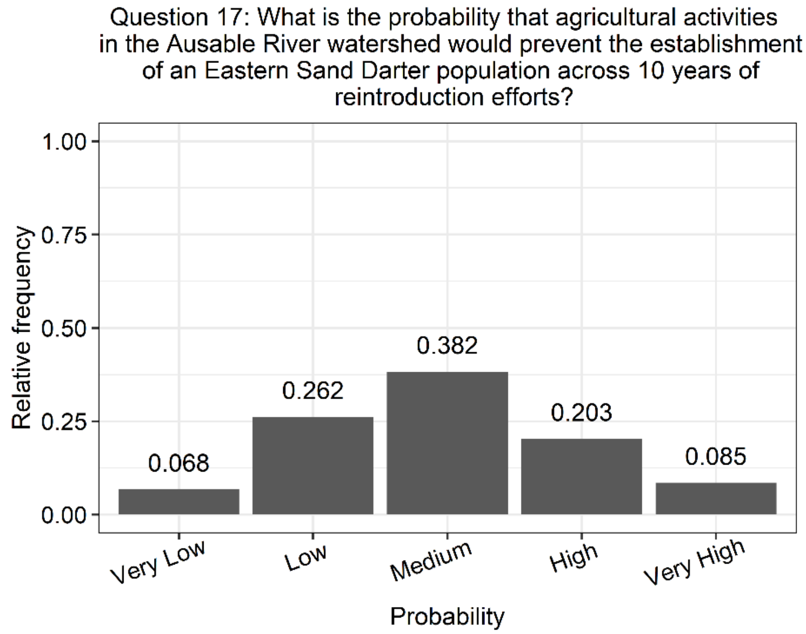

Figure A17. Aggregated participant responses to Question 17: What is the probability that agricultural activities in the Ausable River watershed would prevent the establishment of an Eastern Sand Darter population across 10 years of reintroduction efforts? Responses scaled to 1.

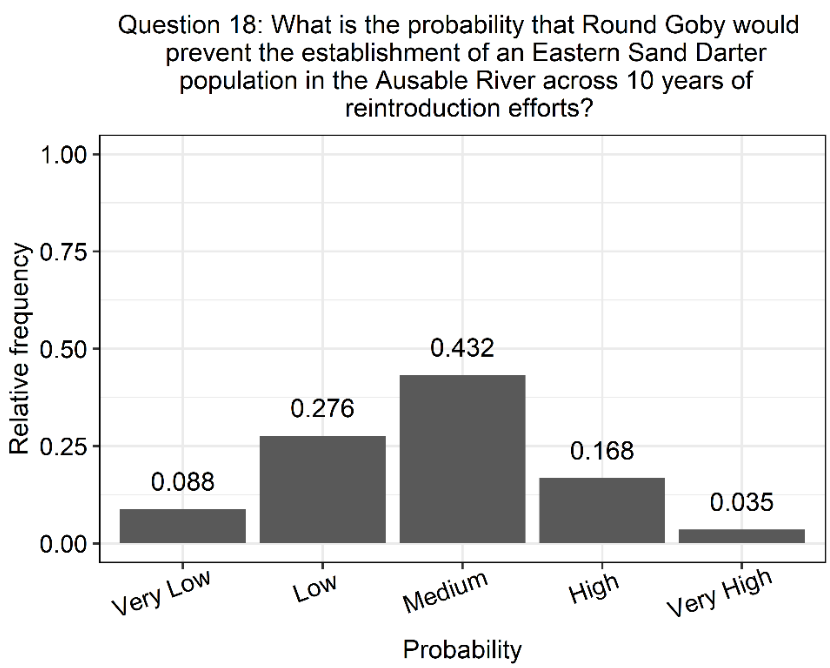

Figure A18. Aggregated participant responses to Question 18: What is the probability that Round Goby would prevent the establishment of an Eastern Sand Darter population in the Ausable River across 10 years of reintroduction efforts? Responses scaled to 1.

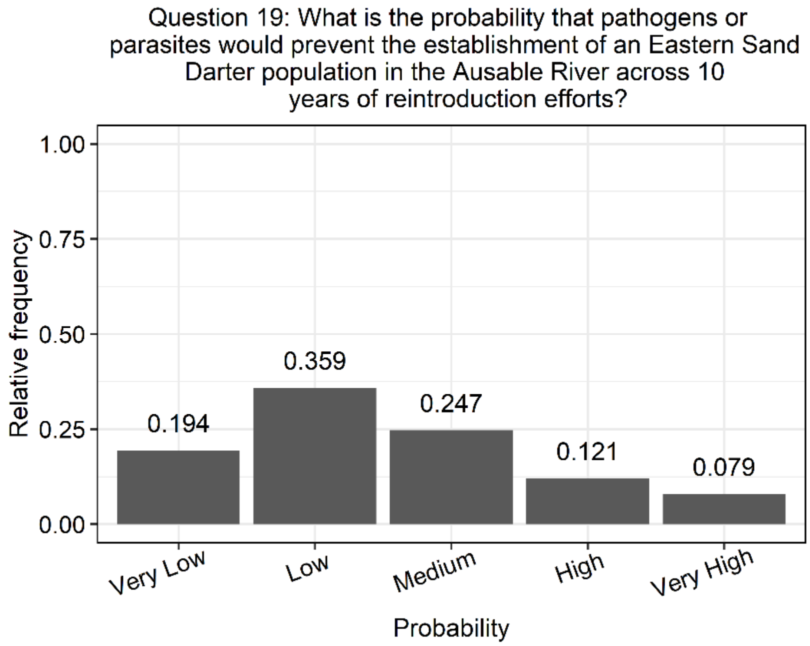

Figure A19. Aggregated participant responses to Question 19: What is the probability that pathogens or parasites would prevent the establishment of an Eastern Sand Darter population in the Ausable River across 10 years of reintroduction efforts? Responses scaled to 1.

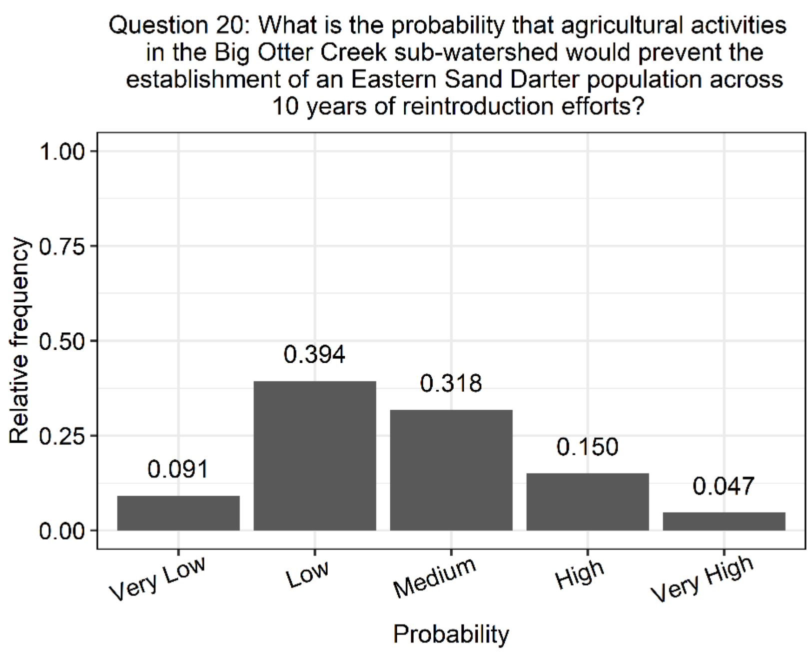

Figure A20. Aggregated participant responses to Question 20: What is the probability that agricultural activities in the Big Otter Creek sub-watershed would prevent the establishment of an Eastern Sand Darter population across 10 years of reintroduction efforts? Responses scaled to 1.

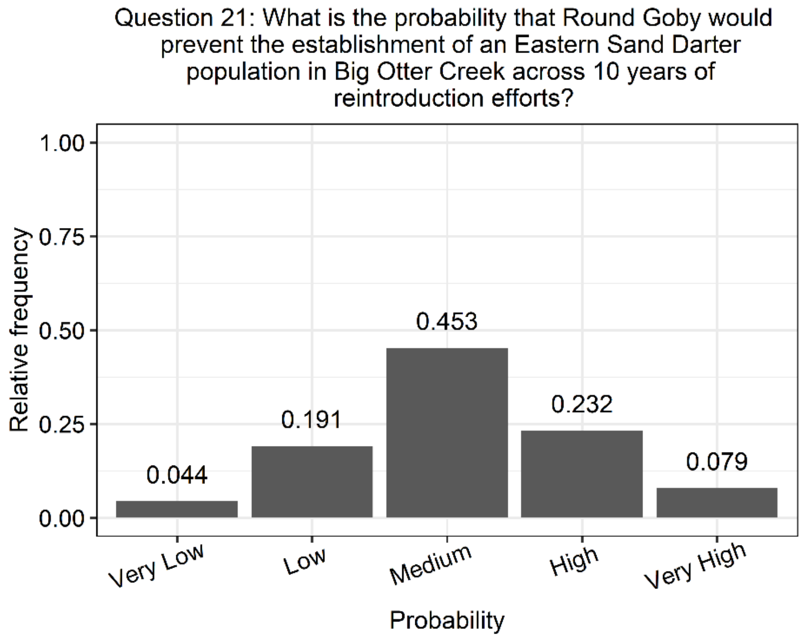

Figure A21. Aggregated participant responses to Question 21: What is the probability that Round Goby would prevent the establishment of an Eastern Sand Darter population in Big Otter Creek across 10 years of reintroduction efforts? Responses scaled to 1.

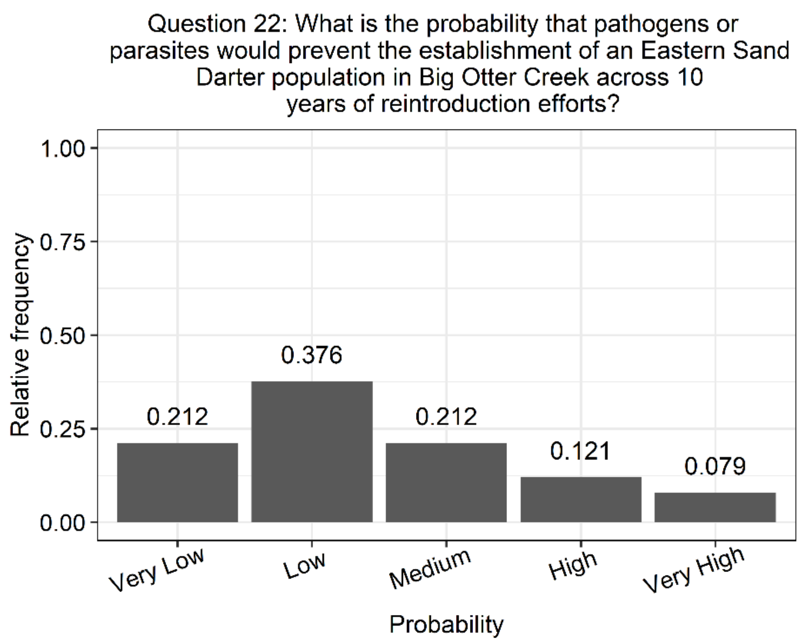

Figure A22. Aggregated participant responses to Question 22: What is the probability that pathogens or parasites would prevent the establishment of an Eastern Sand Darter population in Big Otter Creek across 10 years of reintroduction efforts? Responses scaled to 1.

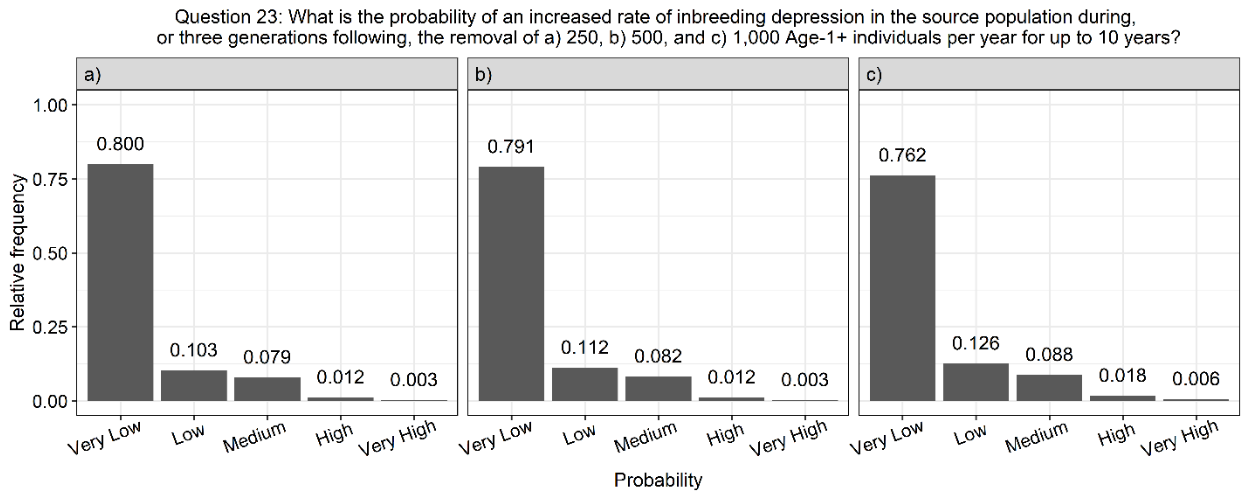

Figure A23. Aggregated participant responses to Question 23: What is the probability of an increased rate of inbreeding depression in the source population during, or three generations following, the removal of a) 250, b) 500, and c) 1,000 age-1+ individuals per year for up to 10 years? Responses scaled to 1.

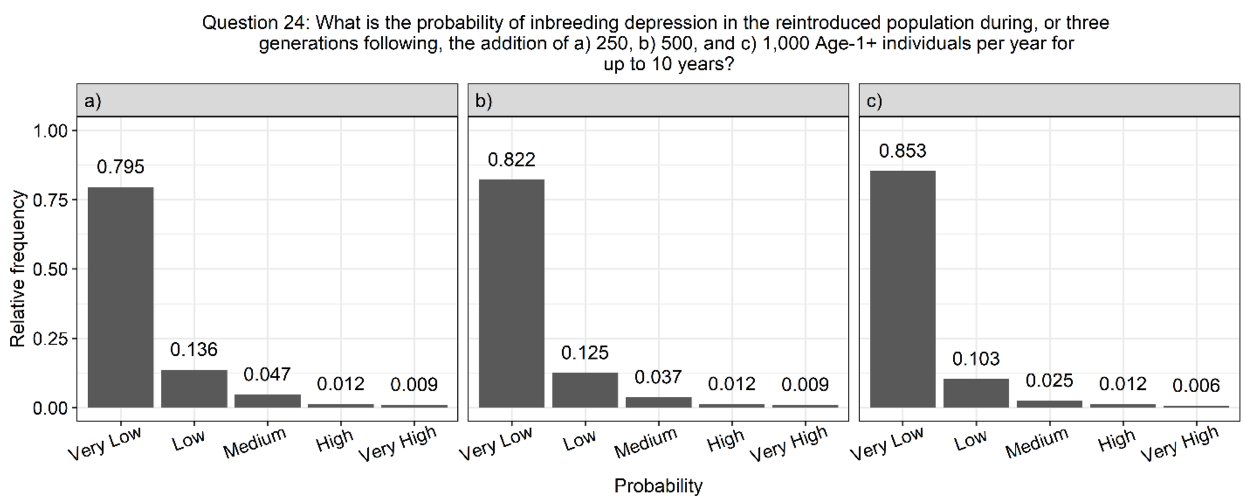

Figure A24. Aggregated participant responses to Question 24: What is the probability of an increased rate of inbreeding depression in the reintroduced population during, or three generations following, the removal of a) 250, b) 500, and c) 1,000 age-1+ individuals per year for up to 10 years? Responses scaled to 1.

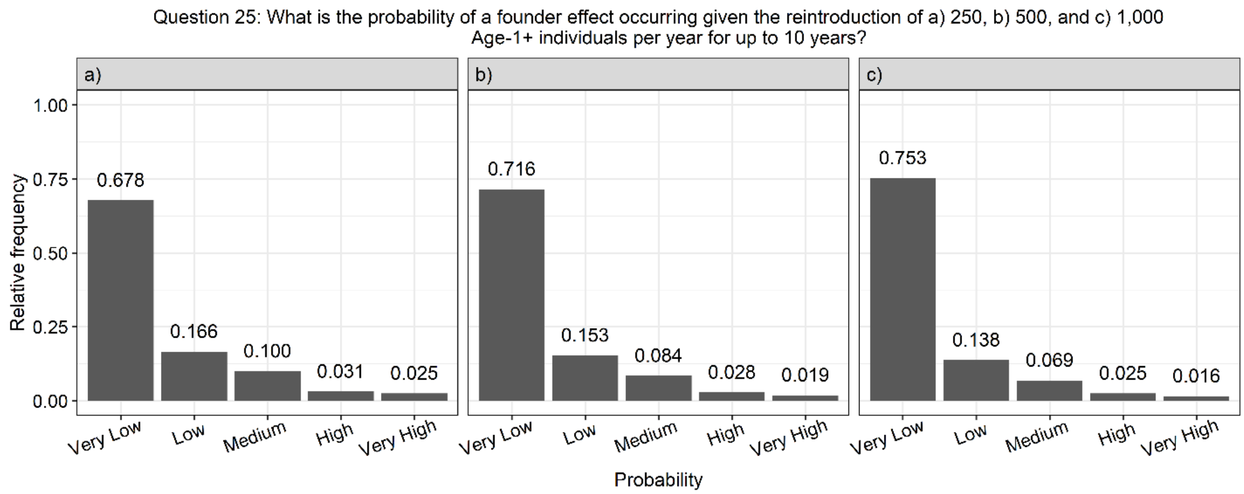

Figure A25. Aggregated participant responses to Question 25: What is the probability of a founder effect occurring in the recipient location given the reintroduction of a) 250, b) 500, and c) 1,000 age-1+ individuals per year for up to 10 years? Responses scaled to 1.

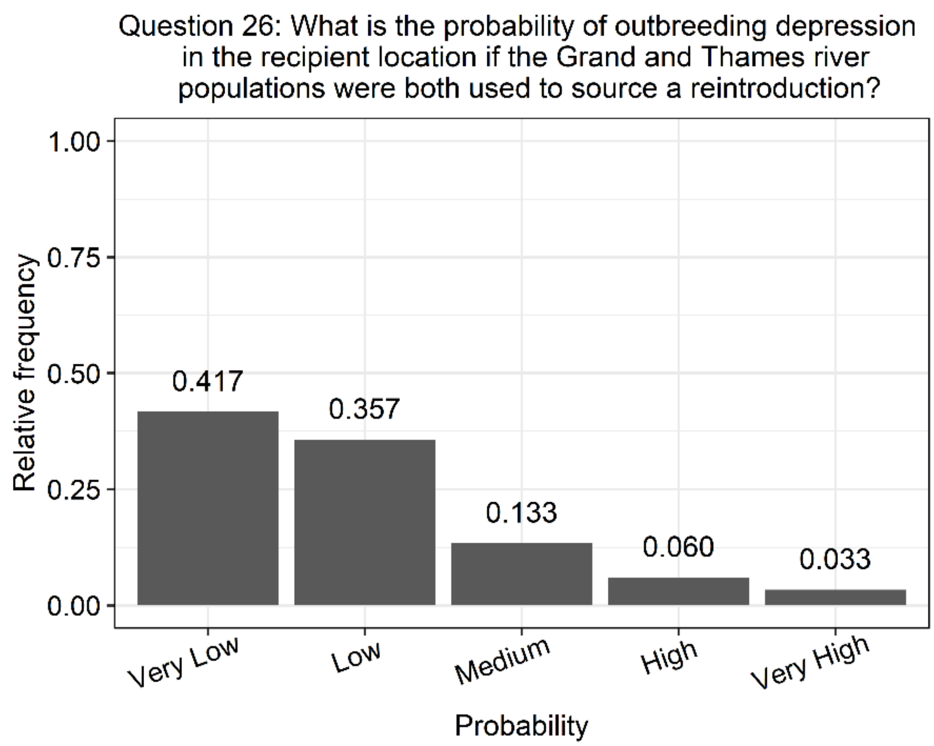

Figure A26. Aggregated participant responses to Question 26: What is the probability of outbreeding depression in the recipient location if the Grand and Thames river populations were both used to source a reintroduction? Responses scaled to 1.

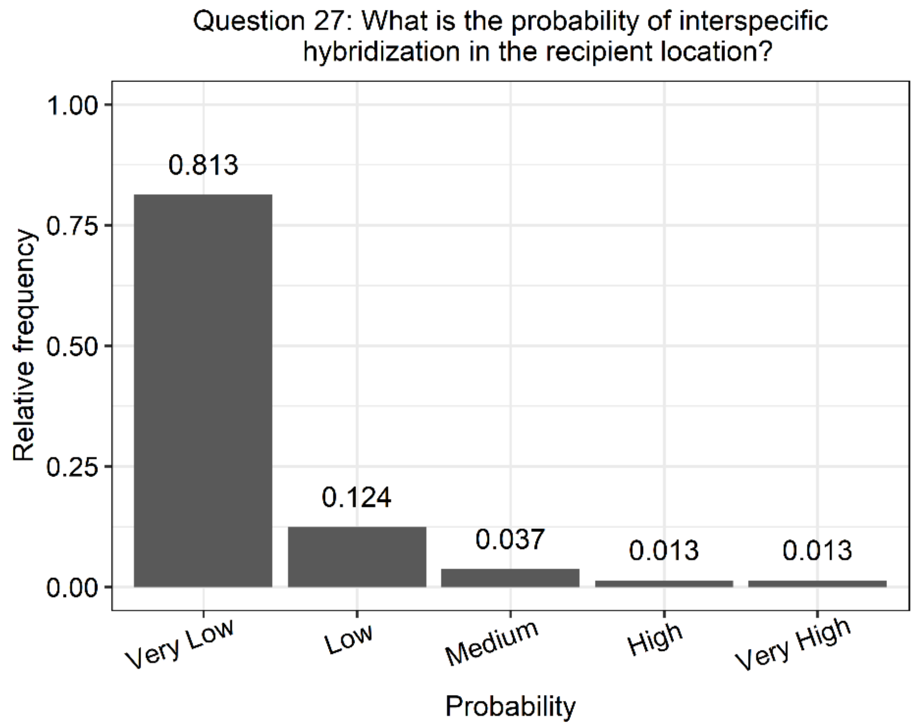

Figure A27. Aggregated participant responses to Question 27: What is the probability of interspecific hybridization in the recipient location? Responses scaled to 1.

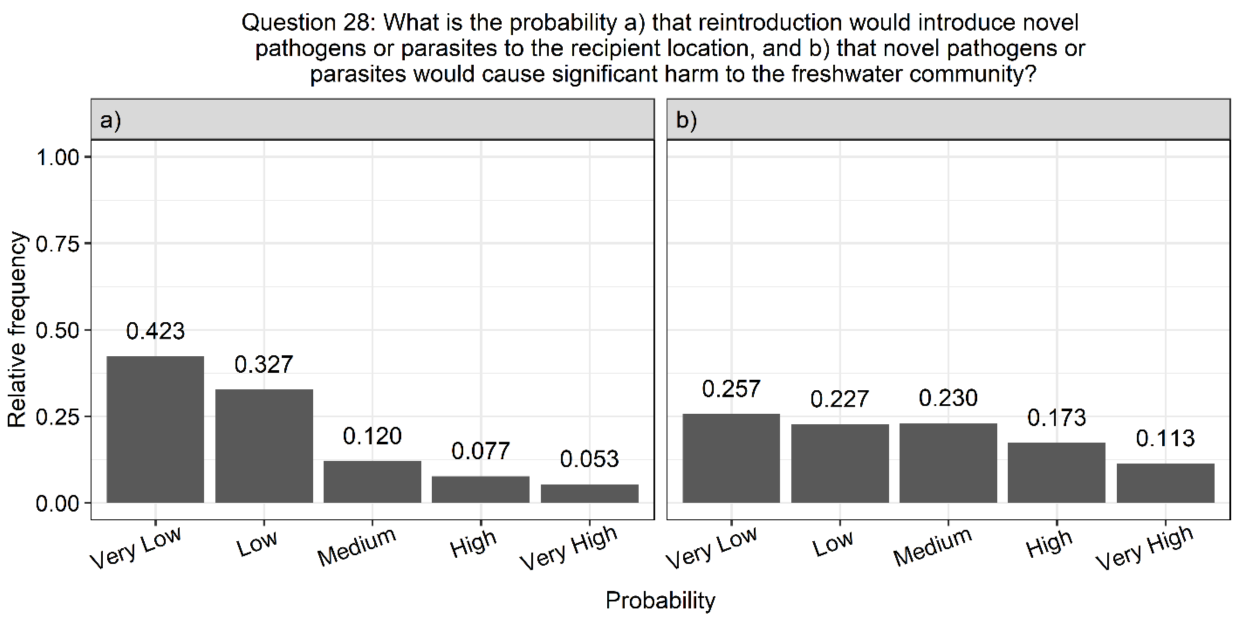

Figure A28. Aggregated participant responses to Question 28: What is the probability a) that reintroduction would introduce novel pathogens or parasites to the recipient location, and b) that novel pathogens or parasites would cause significant harm to the freshwater community? Responses scaled to 1.

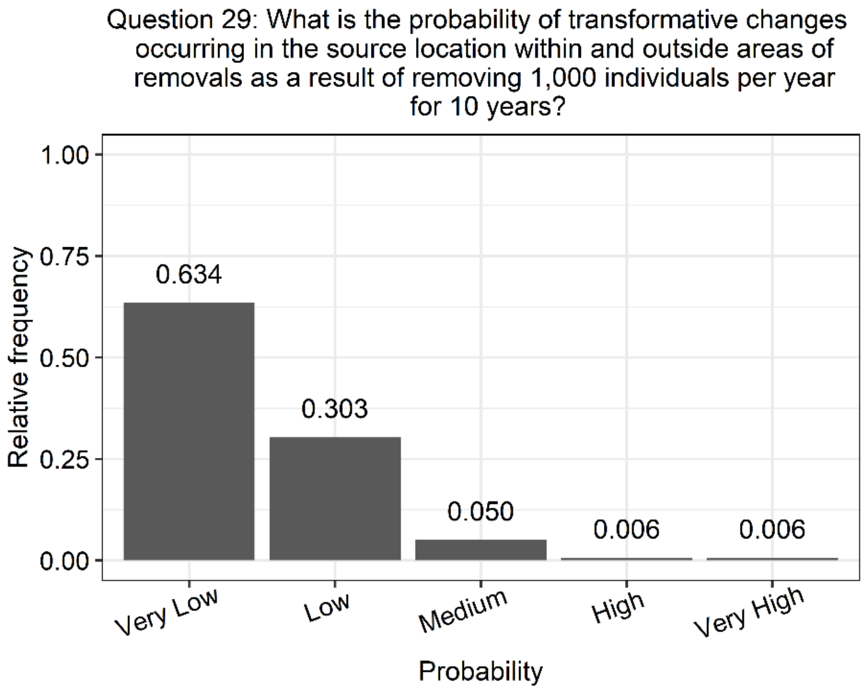

Figure A29. Aggregated participant responses to Question 29: What is the probability of transformative changes occurring in the source location within and outside of areas of removals as a result of removing 1,000 individuals per year for 10 years? Responses scaled to 1.

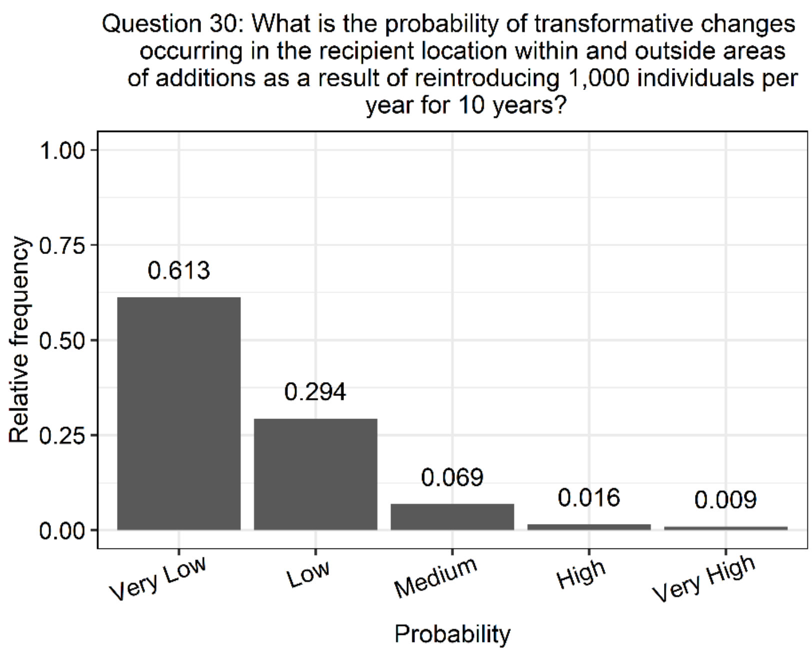

Figure A30. Aggregated participant responses to Question 30: What is the probability of transformative changes occurring in the recipient location within and outside of areas of additions as a result of reintroducing 1,000 individuals per year for 10 years? Responses scaled to 1.