Using eDNA to Elucidate Silver and Bighead Carp Range Expansion in Two Missouri River Tributaries in Eastern South Dakota

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Data Archiving Statement

Data and R code used in this manuscript are available in the GitHub repository: https://github.com/lindseylabrie/carp_eDNA

Author Statement

All authors have read and approved this manuscript, which is represented here solely as a preprint version. It has not been accepted or published elsewhere. The authors declare no competing interests.

Abstract

This study used environmental DNA (eDNA) to detect Bighead Carp (*Hypophthalmichthys nobilis*) and Silver Carp (*H. molitrix*) above and below barriers to fish movement in two tributaries of the Missouri River in eastern South Dakota. The barriers consisted of a natural chain of waterfalls in Sioux Falls in the Big Sioux River and a spillway at the downstream end of Lake Vermillion in the Vermillion River. A total of 213 samples and 38 field blanks were collected over two years (2021/2022). We used filtration methods to collect carp eDNA from the water and qPCR techniques to quantify the amount of eDNA in each sample. The probability of detecting eDNA below the barrier in the Big Sioux River was 51% (95% Credible Interval (CrI): 2 to 97%) and 77% (95% CrI: 8 to 99%) in the Vermillion River. The probability of detecting eDNA above both barriers was significantly smaller: 1% (95% CrI: 0.02 to 24%) for both rivers, with positive above-barrier samples detected only from one sampling date in the Vermillion River. The detection of positive samples above the spillway barrier in the Vermillion River provides the first evidence that Bighead and Silver Carp may have expanded their range to habitats upstream of their documented range in eastern South Dakota. This study demonstrates the utility of using eDNA to detect Bighead and Silver Carp both in areas of known and unknown invasive carp presence in smaller tributary streams to the Missouri River.

Introduction

Bighead and Silver Carp were introduced into the southern United States in the early 1970s as a means of biocontrol of algae in aquaculture ponds and wastewater treatment facilities (Kolar et al., 2005). Soon after their introduction, both species escaped and began establishing populations throughout the Mississippi River basin and beyond (Nico et al., 2023a, 2023b). In

Midwestern rivers, these species have become a threat to many freshwater lotic systems since their arrival in the early to mid 1990s (Kolar et al., 2005).

Bighead and Silver Carp are planktivorous filter feeders which compete with native planktivorous fishes such as Gizzard Shad (*Dorosoma cepedianum*), Bigmouth Buffalo (*Ictiobus cyprinellus*), Paddlefish (*Polyodon spathula*), Bluegill (*Lepomis macrochirus*), and Emerald Shiner (*Notropis atherinoides*), causing varying environmental effects (Freedman et al., 2012; Pendleton et al., 2017; Phelps et al., 2017). In the Missouri River Basin, many barriers limit the upstream movement of both invasive and native fishes. The barriers include dams on the mainstem Missouri and low-head dams and impoundments in the James, Vermillion, and Big Sioux Rivers (Figure 1). Gavin's Point Dam is a major barrier that prevents upstream movement of these carp into upstream sections of the Missouri River, but they may freely swim between the mainstem Missouri and its tributaries in the stretch of river below Gavin's Point Dam.

Streams in eastern South Dakota are on the northwestern-most leading edge of the Bighead and Silver Carp invasion (Hayer et al., 2014). Bighead and Silver Carp first officially appeared in South Dakota in 1998 and in 2003 respectively (Kolar et al., 2005). In the spring of 2019, above-average snowfall followed by rapid melting and early spring rains caused major flooding events throughout rivers in the Midwest (Bagwell & Peters, 2019; Flanagan et al., 2020). In eastern South Dakota, this flooding impacted multiple tributaries to the Missouri River (Francis & Melliger, 2021), causing them to rise to record-high flood stages. The flooding allowed potential pathways for the spread of invasive carp above two perceived barriers to upstream fish movement: a chain of natural waterfalls ("the falls") in Sioux Falls in the Big Sioux River and a spillway at the downstream end of Lake Vermillion in the Vermillion River. In fact, carp of an undetermined species were visually confirmed swimming over the spillway in

the Vermillion River (B. Schall, personal communication, January 2021). The presence of Bighead and Silver Carp above these barriers in the Big Sioux and Vermillion Rivers was previously determined to be nonexistent, but the flooding created new uncertainty surrounding the invasion front in southeastern South Dakota.

Environmental DNA (eDNA) water sampling has been used to detect Bighead and Silver Carp in other systems and has especially targeted barriers to fish passage into the Great Lakes ecosystems (Erickson et al., 2016; Erickson et al., 2017; Jerde et al., 2013; Song et al., 2017), but there is a lack of its implementation in smaller rivers, especially in the Missouri River Basin. eDNA is a beneficial tool to use because physically sampling an organism is not required, therefore reducing operating costs and minimizing disturbance of the ecosystem, while still providing valuable contemporary evidence for the presence of a species (Thomsen et al., 2012). eDNA taken from water samples allows for the detection of organisms in waterbodies where populations are in low or unknown quantities (Lodge et al., 2012). This is especially important when considering invasive species in novel environments (Erickson et al., 2016). Positive eDNA detections of invasive organisms in waterbodies where their status was previously unknown can then trigger management actions to prevent the unchecked spread of the species.

The purpose of this study was to measure the presence and quantity of invasive carp eDNA below and above two major barriers to fish movement in the Big Sioux and Vermillion Rivers (a chain of natural waterfalls and a man-made spillway, respectively) to determine if recent flooding events allowed for Bighead and Silver Carp movement into habitats above those two barriers. We also wanted to evaluate the efficacy of our eDNA sampling regime by 1) determining the number of samples needed to obtain a positive eDNA detections, 2) comparing the effects of filtering method on the amount of eDNA in each sample, 3) comparing the effect of

two different extraction protocols on the overall amount of eDNA in each sample, and 4) determining the overall probability of detecting Bighead and Silver Carp eDNA above each barrier in both rivers. Data collected from this study is intended to help inform local and basin-wide management efforts to control invasive carp populations in eastern South Dakota and beyond.

Methods

Study organisms and sites

The Big Sioux River is a fifth-order stream that begins in Roberts County, South Dakota and flows 674 river kilometers southward through South Dakota to its confluence with the Missouri River on the South Dakota-Iowa border (Omernik, 1987). It is the most populated river basin in South Dakota (Dieterman & Berry, 1999). There exists a major barrier to upstream fish movement in Sioux Falls—a chain of natural waterfalls called Falls Park. The Vermillion River is a fifth-order stream that flows 242 river kilometers from the confluence of the East and West Fork Vermillion Rivers southward through South Dakota to its confluence with the Missouri River on the South Dakota-Nebraska Border (Braaten & Berry Jr, 1997). A large spillway (12.5m high) at the downstream end of Lake Vermillion in the East Fork Vermillion River acts as a potential barrier to upstream fish movement (Hayer et al., 2014). Both the Big Sioux River and Vermillion River experience intermittent patterns of flooding and drought, and flows are highly dependent on the amount of precipitation, including snowmelt, and agricultural runoff (Underhill, 1959).

Sampling methods

Water sampling took place over two field sampling seasons. In the first field season, at each sample location, two water samples were collected using two, 2-Liter high-density

polyethylene (HDPE) bottles. In the second field season, one, 2L sample was taken at each sample location. Prior to sampling, all bottles were sterilized with 20% bleach solution for ≥ 10 seconds and rinsed with distilled water (Coulter et al., 2019). Disposable nitrile gloves were worn during sample collection and were replaced after each sampling location. The most downstream location in each river was sampled first, and each successive sample was collected upstream of the previous sample to avoid eDNA cross-contamination. All samples were collected while standing on the shoreline to reduce the risk of boots or waders contaminating the water with invasive carp eDNA. One negative control sample (one, 2-L bottle of distilled water) was collected above and below each barrier. After sampling, all bottles were placed in coolers on ice until filtration was carried out no more than 8 hours after sample collection.

The water samples were filtered through 1.5 µm glass microfiber filters (Cytiva Whatman Binder-Free Glass Microfiber Filters; grade 934-AH circles; diameter outside: 47mm; borosilicate glass; grade 934-AH filter; 47 mm circle) (Eichmiller et al., 2014) using a magnetic polyphenylsulfone filter funnel (Eichmiller et al., 2014; Nukazawa et al., 2018; Coulter et al., 2019). All supplies were sterilized with 20% bleach for ≥ 10 seconds, rinsed with distilled water, and dried with a new paper towel between samples. Water samples were drawn through the filter until the pores were clogged and water could no longer pass through the filter, and the volume of water that passed through was recorded. Each filter was stored in a separate 95% ethanol in a 15 mL polypropylene Falcon tube at -20°C until eDNA extraction could occur.

eDNA extraction

Prior to extraction, filters were cut in half, with one half returned to the 15 mL Falcon tube in the freezer and the other half cut into three smaller pieces. The three pieces were placed in 1.5 mL microcentrifuge tubes and allowed to air dry in a fume hood over a period of ≥ 4 hours

until all ethanol had evaporated. Tools were sterilized with 20% bleach for ≥ 10 seconds, rinsed with distilled water, and then rinsed with nanopure water to prevent contamination between filters. eDNA extraction was performed in a designated sequencing room at the University of South Dakota and according to the Qiagen DNeasy Blood and Tissue Extraction protocol (Qiagen, 2023) with one modification at the end step to centrifuge 100 µL of elution buffer through the spin columns twice instead of 200 µL once (i.e., "Kristie" protocol (Schmidt et al., 2021)). Extracted samples were stored in 1.5 mL or 2 mL microcentrifuge tubes at -20 °C.

Quantitative Polymerase Chain Reaction (qPCR) analysis

The real-time quantitative polymerase chain reaction (qPCR) analysis was conducted using an Applied Biosystems™ QuantStudio™ 3 Real-Time PCR System thermocycler (Thermo Fisher). The forward primer, reverse primer, and gBlock used in this study were developed by Erickson et al. (2017) and targeted the mitochondrial gene *nd5*, where the primer sequence binds to an area of the mitochondrial genome that is conserved between both Bighead and Silver Carp, and the probe sequence binds to an area of the mitochondrial genome that is specific to either species (Erickson et al., 2017). Each well had a volume of 20 µL, containing 7 µL molecular grade water, 10 µL TaqMan™ Fast Advanced Master Mix (Thermo Fisher), 1 µL of 10 nanomole forward and reverse primer mix, 1 µL of 5 nanomole probe mix, and 1 µL of extracted eDNA sample. A 313-base pair Megamer ssGene Fragment, or gBlock, containing DNA sequences conserved between both Bighead and Silver Carp was used to create a standard dilution series which served as the eDNA calibration curve for each qPCR run (Erickson et al., 2017). The 96-well plates contained four replicates of each of the following: eight field samples, one field blank, one extraction blank and one positive control. Positive controls were extracted with the DNeasy Qiagen Blood and Tissue Kit from Bighead and Silver Carp tissues. Each plate

also contained three spiked replicates of each sample (extracted sample plus 0.1 μL of the 10^3 dilution per well). Ten, no-template control wells (1 μL nanopure water) and two replicates of a 10^6 standard dilution series derived from the G-block megamer were included on each plate (Table 1). qPCR cycling conditions followed the protocol in Erickson et al. (2017). We considered each sample to have been positive if at least one sample replicate amplified during qPCR, and negative if none of the replicates amplified.

Pilot study

First, to evaluate the number of water sample replicates needed to obtain a positive detection in areas of known Bighead and Silver Carp presence, we took five, 2-L samples in September 2022 at one location in the Big Sioux River (42.495167, -96.477236) and one location in the Vermillion River (42.772914, -96.930374), where the USFWS was contemporaneously performing their eDNA sampling and Dozer trawling for invasive carp. Second, to determine if eDNA quantities varied when following the Qiagen extraction protocol versus the modified extraction protocol (i.e., "Kristie" protocol; Schmidt et al., 2021), we extracted one half of each filter using the Qiagen protocol and the other half using the "Kristie" protocol (Schmidt et al., 2021) and then analyzed the samples.

Statistical analysis

We evaluated whether filtering method (i.e., field vs lab) caused a difference in the probability of eDNA detection. We used Program R (version 4.1.1; R Core Team 2022) and the *brms* package (Bürkner, 2017) to fit a Bayesian generalized linear mixed model with a Bernoulli likelihood with a logit link with the following model and default priors:

$$E_i \sim \text{Bernoulli}(p_i),$$

$$\text{logit}(p_i) = \alpha + \boldsymbol{\beta}\mathbf{X} + \gamma_{\text{sample_name}}$$

Where E_i is the presence or absence of eDNA in sample $_i$, p_i is the probability of detection, α is the intercept, $\boldsymbol{\beta}\mathbf{X}$ is a vector of 3 parameters representing two predictors ($\mathbf{X}$ = filter_method, species) and their interaction and γ_{sample_name} is the random intercept for sample name.

To evaluate the probability of detection as a function of species, river, and location in respect to the barriers in each river, we again used a Bernoulli likelihood with a logit link using the following equation:

$$E_i \sim \text{Bernoulli}(p_i),$$

$$\text{logit}(p_i) = \alpha + \boldsymbol{\beta}\mathbf{X} + \gamma_{sample_name} + \gamma_{species}$$

$$\alpha \sim \text{Normal}\ (-10,1)$$

$$\boldsymbol{\beta}\mathbf{X} \sim \text{Normal}\ (0,1)$$

$$\gamma \sim \text{Student-t}(3, 0, 2.5)$$

Where E_i, p_i, and α are as defined above, $\boldsymbol{\beta}\mathbf{X}$ is a vector of 7 parameters representing three predictors (quantity, location, river) and their interactions, and γ_{sample_name} and $\gamma_{species}$ are the random intercepts for sample name and species, respectively.

Both models were run using a Hamiltonian Monte Carlo No-U-Turn sampler with 4 Markov chains, 2,000 iterations per chain with a warm-up of 1,000 iterations. We evaluated the fit of the model by running posterior predictive checks and determined it properly converged if Gelman-Rubin statistics were less than 1.1 (Brooks & Gelman,1998). We sampled from the posterior and estimated the relationships for the model and predicted the mean with 95% credible intervals. We ran a sensitivity analysis to assess the impact of the priors on our presence/absence model by doubling the standard deviation values to Normal (-10,2) for the intercept, Student's t (3, 0, 5) for the random intercepts, and Normal (0,2) for the beta values.

We also visualized how other factors may have affected the quantity of eDNA in our samples using *ggplot2* (Wickham et al., 2016), such as the length of time between sample collection and filtering, sample month, the volume of water filtered, the number of days between filtering and extraction, and the number of days between extraction and qPCR.

Results

Water sampling

A total of 213 water samples and 38 field blanks were collected after two years of field sampling. (98 water samples and 20 field blanks in 2021, 115 water samples and 18 field blanks in 2022). In 2021, one-half of the water samples were filtered in the field using a 7.2-liter, manual pump MityVac MIT7400 fluid evacuator (Figure S-1a). The other half of the samples were transported back to the lab and filtered using a 1000 mL Büchner flask (Figure S-1b). In 2022, samples were exclusively filtered using the field filtering method. The average filtered volume was 382 mL (range 100 to 1650 mL). Each year, samples were collected from sampling sites in both rivers three separate times. In 2021, Big Sioux River samples were collected from three below-barrier sites and six above-barrier sites in three sampling sessions. This was expanded to five below-barrier sites and eleven above-barrier sites in 2022 (Figure S2). In 2021, Vermillion River samples were taken at two below-barrier sites and seven above-barrier sites. This was expanded to four below-barrier sites and fourteen above-barrier sites in 2022 (Figure S3).

Invasive carp were observed swimming below the barriers at almost all sampling sessions, except for the below-barrier Vermillion River samples in October and September 2021, where only fish carcasses of various species, including Bighead and Silver Carp, were observed

in the water below the spillway. In 2022, we visually observed carp of undetermined species at all water sampling sessions below both barriers.

qPCR results

Bighead and Silver Carp eDNA was detected below both barriers in 2021 and 2022 in varying quantities (Table 3). In July 2022, Bighead and Silver Carp eDNA was detected in two and three samples respectively above the Lake Vermillion spillway (Figure S4), but not in previous or successive samples.The probability of detecting eDNA below the barrier in the Big Sioux River was 51% (95% Credible Interval (CrI): 2 to 97%) and in the Vermillion River was 77% (95% CrI: 8 to 99%). The probability of detecting eDNA above the barriers was significantly smaller: 1% (95% CrI: 0.02 to 22%) in the Big Sioux River and 1% (95% CrI: 0.02-24%) in the Vermillion River. The probability that detection was higher below the barriers than above the barriers was greater than 99% (Figure 2).

There was little difference between the mean quantity of eDNA obtained from field and lab filtering methods, with less than one-hundredth of one percent difference between field and lab filtered samples (0.0003%, 95% CrI: 0.001 to 0.6%) (Figure 3). This was the basis for our choice to only perform field filtration in the second sampling season. Through pilot study sampling, we determined that one 2L water sample at each sampling location was sufficient to obtain a positive detection for both Bighead and Silver Carp in both rivers, with a 10% probability of false negative detection. The probability of false negatives halved when more samples were taken at each location (i.e., 5% for two samples per location, 2.5% for three samples per location, etc.) The extraction protocol type (i.e.,“Qiagen” versus “Kristie”) appeared to have an impact on mean eDNA quantities, which reinforced our decision to follow the modified extraction protocol described in the methods section above. Other factors such as the

length of time between sample collection and filtering, sample month, the volume of water filtered, the number of days between filtering and extraction, and the number of days between extraction and qPCR did not appear to have any strong effect on the amount of eDNA detected with qPCR (Supplementary Figures 5-9).

Model performance

Both models performed well, and observed data was within the predicted range of the posterior estimates. Trace plots indicated proper mixing of the Markov chains, all R-hat values were < 1.001, and all parameter estimates had large effective sample sizes. The Bayesian R^2 value for the filter method model was 0.82 (95% credible interval (CrI): 0.73 to 0.89) and for the presence/absence model was 0.76 (95% CrI: 0.67 to 0.84). Posterior predictive checks (Berkhof et al., 2000) generally replicated the shape of the data for both models. The sensitivity analysis showed model parameter estimates were not sensitive to the priors (Figure 4).

Discussion

This study was the first to use eDNA to detect Bighead and Silver Carp above barriers to upstream movement in Missouri River tributaries in eastern South Dakota. We were able to demonstrate the utility of the filtration method to quantify eDNA from water samples within these two rivers. The filtration method used in this study has the potential to be more accessible than other eDNA sampling methods. The manual pneumatic pump and magnetic filter funnel are relatively inexpensive, easily portable, and can be deployed on-site and allow for immediate filtration upon water sample collection. We determined that filtering water from one 2-L sample was sufficient to detect eDNA at least 90% of the time when both species of carp are present. The number of days between filtration and extraction, or the number of days between extraction

and qPCR analysis did not appear to affect the quantity of eDNA detected in each positive sample.

The probability of detecting eDNA in our samples below the barriers was high (>54%) and the probability above the barriers was low but greater than zero (<1%) in both rivers. We obtained three positive eDNA detections above the spillway in the Vermillion River, within Lake Vermillion in June 2022, but not in previous or successive samples. We are confident that positive samples above the spillway were true positives because there was no evidence of eDNA contamination in the field blanks or qPCR no-template controls. There have been no official reports of invasive carp above the spillway, however it is possible that invasive carp have been persisting in Lake Vermillion in relatively low numbers and that we simply did not sample in areas where they were present during the other rounds of sampling. The presence of boats on Lake Vermillion during our July 2022 sampling session could explain the positive detections above the spillway barrier. Invasive carp DNA could have sloughed off the hull of a boat that had previously been in carp-positive waters, resulting in positive detections. Another explanation is that invasive carp initiate spawning movements in early summer when the hydrograph begins to rise (Erickson et al., 2016). Because eDNA has short persistence times in freshwater ecosystems (Eichmiller et al., 2014), it is possible that the invasive carp made an upstream spawning run out of the lake and into the Vermillion River immediately prior to sampling. Both could offer potential explanations for the positive samples above the barrier within the lake.

South Dakota is predicted to experience an increase in climate extremes, including persistent severe droughts, at least a 15% increase in annual precipitation, and an increase in the intensity and interval of severe flooding (Frankson et al., 2022). The effects of flooding have been evident in the recent past during the flood of 2019, when Falls Park became inundated with

water and the spillway at Lake Vermillion became compromised, thus providing potential pathways for invasive carp to pass barriers to fish movement. Future eDNA water sampling or physical sampling methods (i.e., capture of live individuals via electrofishing) will be required to confirm the presence of live invasive carp above the barriers in both rivers. Water sampling could be expanded to other small tributaries to assess the presence of these fish in finer detail throughout eastern South Dakota. It may also be necessary to eventually reconsider the effectiveness of these barriers during flooding events.

Conclusion

This study provides evidence that eDNA is a useful technology to implement in smaller tributary systems on the leading edge of the current invasive carp range. We determined that filtering one, 2-L sample per site provided a true positive eDNA detection at least 90% of the time. We established a baseline for fish presence above barriers in the Big Sioux and Vermillion Rivers, however, we recommend continued monitoring to increase the level of certainty of fish presence above the barriers in both rivers, especially in the Vermillion River. We recommend future eDNA water sampling to further elucidate the status of these species above the barriers, coupled with physical sampling efforts to gauge invasion presence and risk into the future.

Acknowledgements

We thank T Devine and J Larsen for their assistance with water sampling and processing, and K Schmidt for demonstrating the qPCR protocol. Dr. A Coulter provided comments and revisions to the manuscript. Funding for this study was provided South Dakota Game Fish and Parks (Grant number F-16-R-1) and the United States Fish and Wildlife Service (Grant number F20AP11067-00). This work was conducted under an approved protocol from the University of South Dakota's Institutional Animal Care and Use Committee (protocol # 10-08-20-23D).

Tables

Table 1: A general 96-well plate set up for detecting Bighead and Silver Carp eDNA. “NTC” stands for “no template control.”

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Field blank	Extraction blank	10^6	10^6
B	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Field blank	Extraction blank	10^5	10^5
C	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Field blank	Extraction blank	10^4	10^4
D	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Field blank	Extraction blank	10^3	10^3
E	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	10^2	10^2
F	Spiked sample 1	Spiked sample 2	Spiked sample 3	Spiked sample 4	Spiked sample 5	Spiked sample 6	Spiked sample 7	Spiked sample 8	Spiked field blank	Spiked extraction blank	10^1	10^1
G	Spiked sample 1	Spiked sample 2	Spiked sample 3	Spiked sample 4	Spiked sample 5	Spiked sample 6	Spiked sample 7	Spiked sample 8	Spiked field blank	Spiked extraction blank	Positive control	Positive control
H	Spiked sample 1	Spiked sample 2	Spiked sample 3	Spiked sample 4	Spiked sample 5	Spiked sample 6	Spiked sample 7	Spiked sample 8	Spiked field blank	Spiked extraction blank	Positive control	Positive control

Table 2: Average eDNA quantification (copies/μL) for each river, above and below barriers, for each species in both years.

2021			
		Bighead Carp	Silver Carp
Vermillion River	Above	0	0
	Below	555	0.009
Big Sioux River	Above	0	0
	Below	18.4	1.54

2022			
		Bighead Carp	Silver Carp
Vermillion River	Above	**0.302**	**0.00960**
	Below	24.1	1.53
Big Sioux River	Above	0	0
	Below	2.74	0.391

Figures

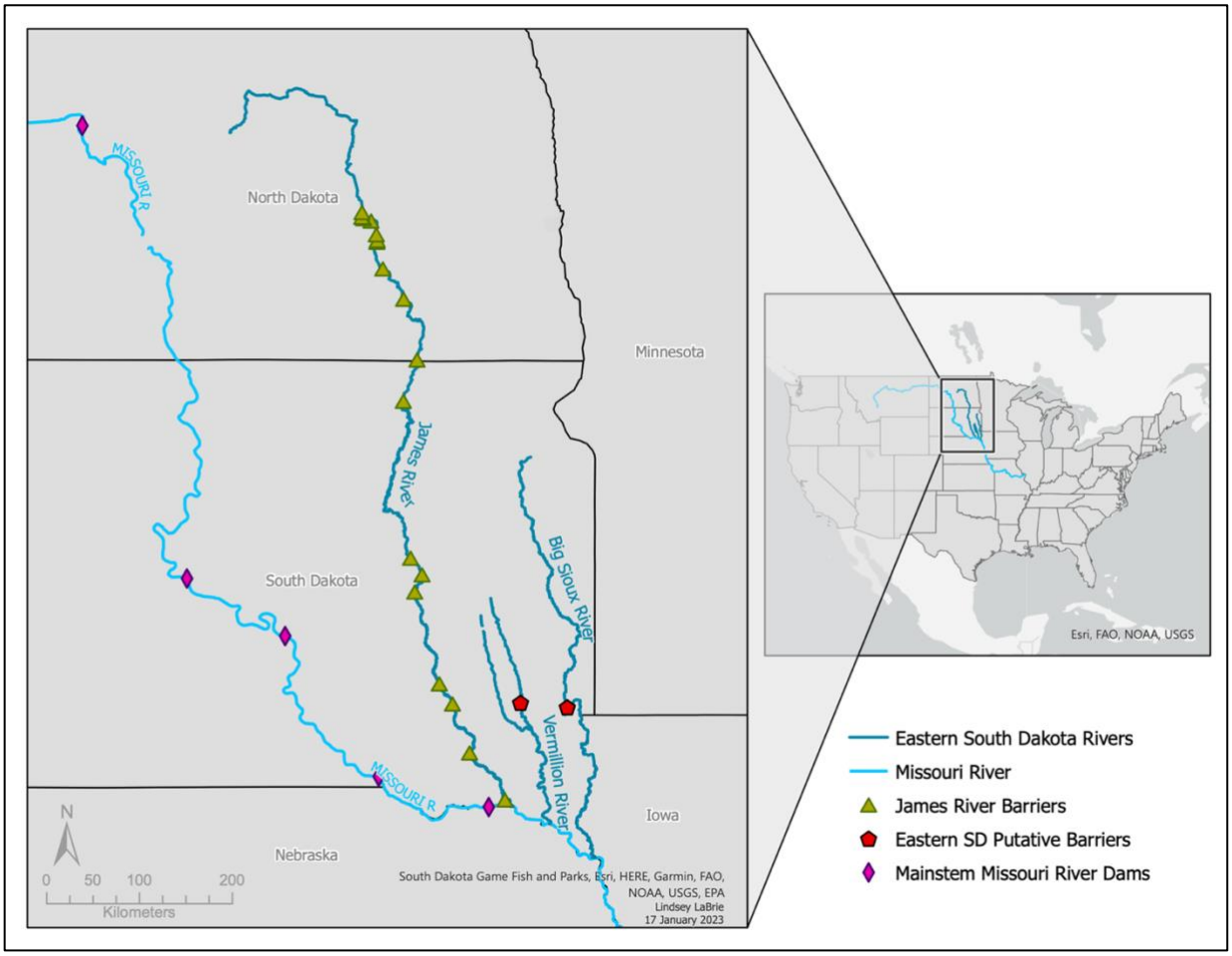

Figure 1: A map of barriers to upstream fish movement in the Missouri, James, Vermillion, and Big Sioux Rivers. Dams on the mainstem Missouri River are shown as purple diamonds, poor barriers to fish movement such as low-head dams and rock impoundments in the James River are shown as green triangles (not all barriers are shown at the current map scale), and the putative barriers in the Big Sioux and Vermillion Rivers are shown as red pentagons.

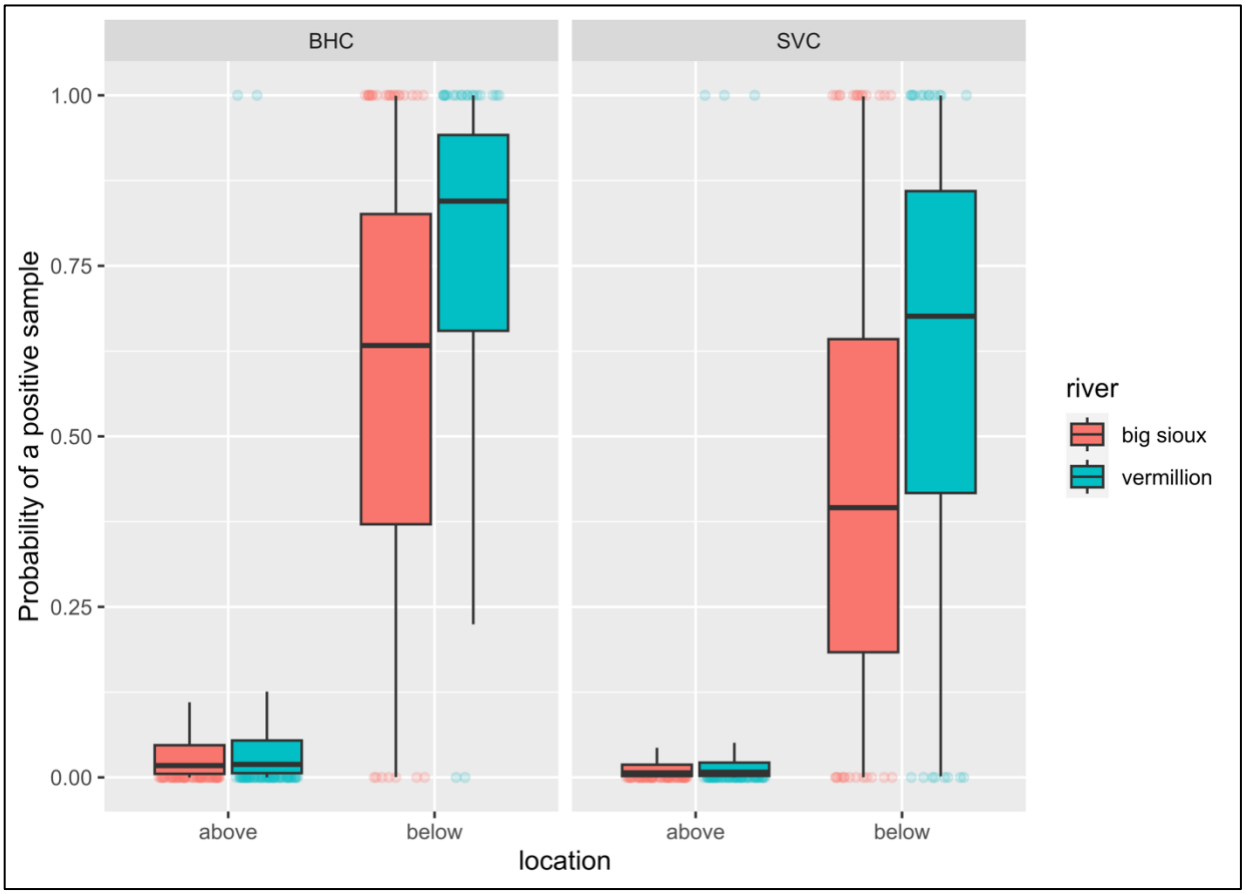

Figure 2: Posterior predictions for the probability of a positive sample for Bighead Carp (BHC) and Silver Carp (SVC) in the Big Sioux and Vermillion Rivers above and below the major barriers to upstream fish movement. The hollow, colored circles represent data points, with zero (0) on the Y-axis indicating no eDNA detection and one (1) on the Y-axis indicating a positive eDNA detection. The probability of a difference between above and below barrier samples was greater than 99%.

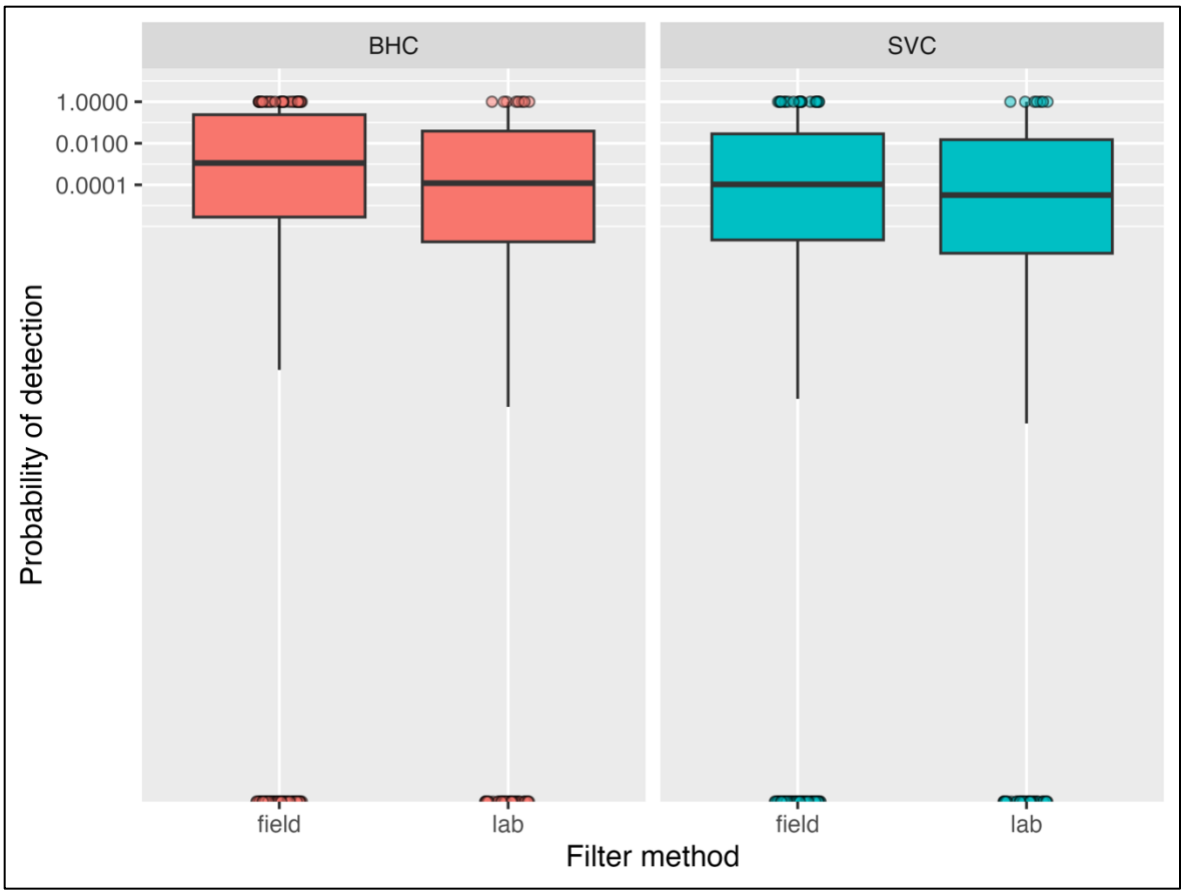

Figure 3: Estimated posterior probability of detecting eDNA from Bighead Carp (BHC) and Silver Carp (SVC) in both rivers (combined) for field and lab filtered samples. The colored circles represent actual data points, with zero (0) on the y-axis indicating no eDNA detection and one (1) on the y-axis indicating a positive eDNA detection. Note that the y-axis is on a log(10) scale.

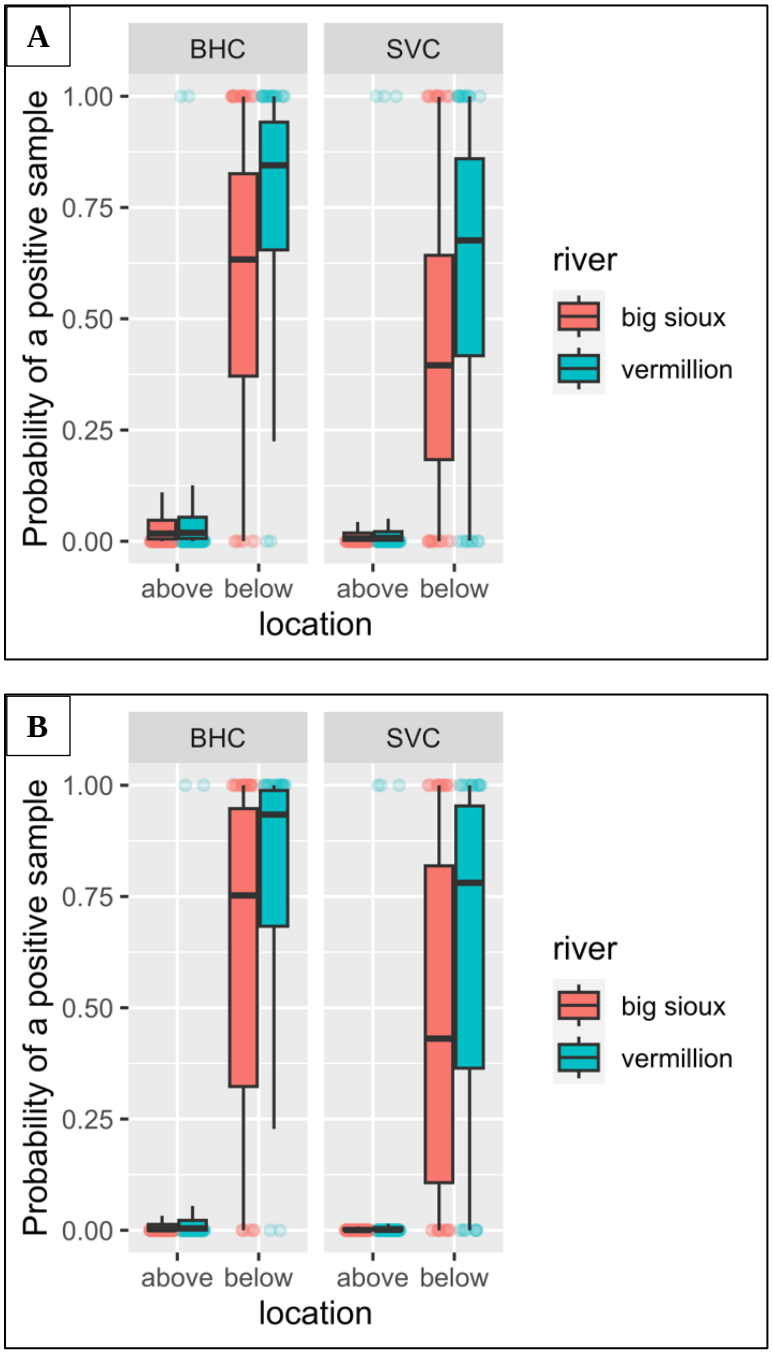

Figure 4: A comparison of the original model estimates (A) and the sensitivity analysis (B) for which the standard deviation of each prior was doubled. The colored circles represent actual data points, with zero (0) on the y-axis indicating no eDNA detection and one (1) on the y-axis indicating a positive eDNA detection.

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