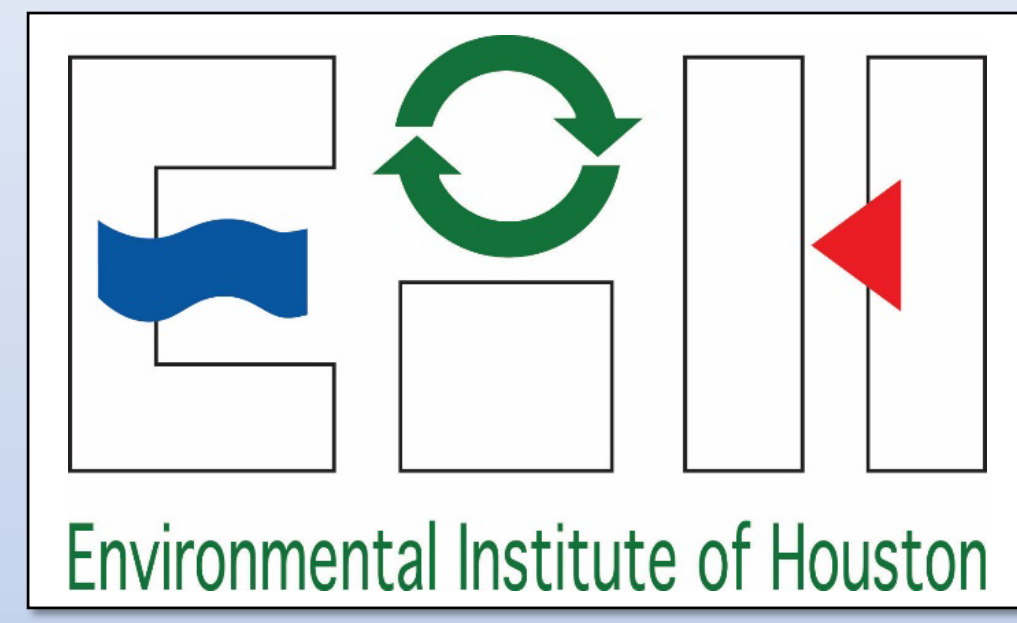


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# Evaluation of the use of eDNA metabarcoding to assess fish assemblage in the rivers and streams of Texas



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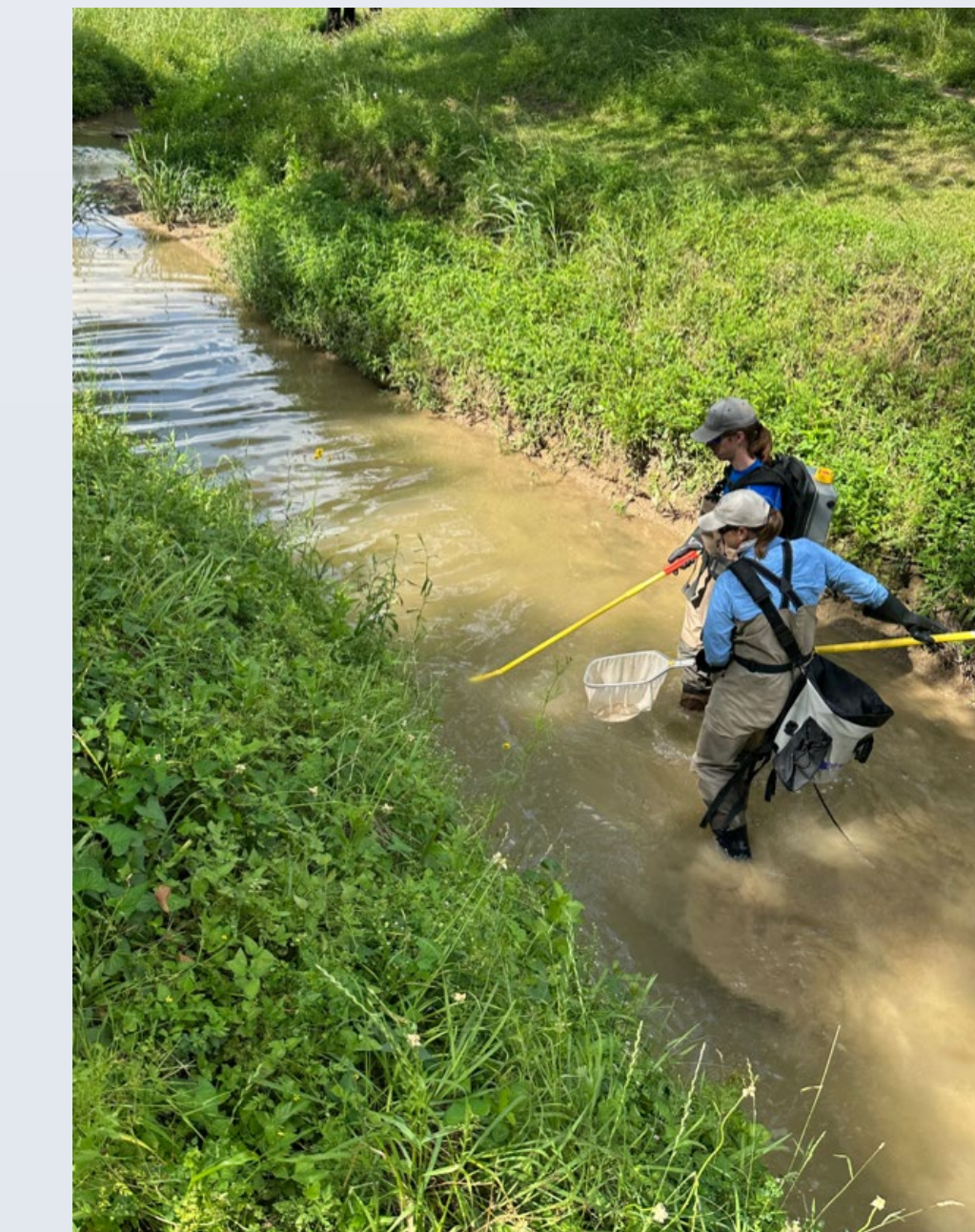


## Introduction

- Fish are an ecologically important part of an aquatic ecosystem, and which kinds and how many of each are present helps to evaluate the health of a river or stream.
  - Conventional methods such as electrofishing and seining are widely used to monitor fish assemblages, but use of environmental DNA (eDNA) metabarcoding is becoming a helpful tool to compliment these conventional methods.
  - Metabarcoding genetically matches up specific segments of the DNA to a known fish database – resulting in a list of species that had DNA present in the water you sampled.
  - The extent and limitations of eDNA metabarcoding are still being investigated with limited but expanding use in governmental monitoring within the U.S.
  - This study is aimed at measuring how the use of eDNA metabarcoding compares to conventional methods across the diverse sampling locations of the National Rivers and Streams Assessment in Texas.
- Objectives of this study are to:
- Characterize the fish assemblages in rivers and streams of Texas.
  - Compare fish assemblage results from electrofishing (or seining where electrofishing is not possible) and eDNA metabarcoding.
  - Evaluate how variables (such as stream order, width, depth, discharge, ecoregion, watershed size, land use/land cover, etc.) in river systems impact the applicability of eDNA metabarcoding.
  - Evaluate the ability to use read counts to infer relative abundance or biomass of fish species detected use eDNA compared to conventional methods.

## Methods

- Reach of sampling is determined by wetted width of river. Total reach = mean wetted width × 40, and is divided into 10 equal segments, with 11 transect lines. The X (designated) site is the center (Figure 2, Figure 3).
- Environmental conditions
  - Water: Dissolved oxygen, temperature, pH, conductivity.
  - Physical characteristics: depth, width, canopy, flow, anthropogenic influence, substrate, slope, bank angle, vegetation, fish cover, and channel constraint.
- eDNA
  - Ambient water grabs will be collected across the sample reach at specific locations for eDNA metabarcoding (Table 1).
  - Samples will be taken at 0.3 M below the surface.
  - All eDNA samples will either be filtered then frozen, or directly frozen after collection (Table 1).
- Conventional methods
  - Electrofishing will be used as the conventional method unless ESA listed species are known to be present, or conductivity is too high.
  - Small rivers/streams (≤ 12m): the entirety of reach and stream will be fished.
  - Large rivers/streams (≥ 13m): Sampling will be concentrated near the bank, switching from left to right bank every 2 segments sampled.
  - Fish will be identified to species and enumerated in size categories before they are returned to the river.
- Analysis
  - All eDNA analysis will be performed using the 12S and 16S mitochondrial genes.



## Benefits of using eDNA

- Easy to collect
- Detection of rare or cryptic species (Ficetola et al 2008)
- Save time in the field
- Causing minimal disturbance to fish and ecosystem

## Limitations of using eDNA

- Variability in DNA persistence
- May include influences from upstream of the reach
- No data on specific characteristics (e.g. size, sex)
- More room for errors in lab, and analysis
- No historical data with eDNA to compare from previous studies

## eDNA samples

Table 1: eDNA grabs to be taken at the sites sampled

Sample	Same day filter	Volume collected		Sample location		
		100 mL	1000 mL	X site samples	Every transect	Live well
Composite	x	x			x	
Single grab (a)	x		x	x		
Single grab (b)			x	x		
Known biomass	x		x			x

## eDNA samples will help us to:

- Look at methodology through our single grab samples, comparing how filtering early affects the quality of DNA and reads we obtain.
- Compare single grab to the composite sample to conventional methods.
- Compare the results of the eDNA composite sample to using the live well with known size and count of fish, to better understand the ability to use read counts of DNA to predict biomass or relative abundance in a river.
- Evaluate the applicability of eDNA for future large-scale fish monitoring programs.

## Literature Cited

- Ficetola, G. F., Miaud, C., Pompanon, F., & Taberlet, P. (2008). Species detection using environmental DNA from water samples. *Biology letters*, 4(4), 423-425
- United States Environmental Protection Agency (USEPA). 2022a. National Rivers and Streams Assessment 2023/24: Field Operations Manual – Wadeable. EPA-841-B-22-006. U.S. Environmental Protection Agency, Office of Water Washington, DC.
- United States Environmental Protection Agency (USEPA). 2022b. National Rivers and Streams Assessment 2023/24: Field Operations Manual – Non-Wadeable. EPA-841-B-22-007. U.S. Environmental Protection Agency, Office of Water Washington, DC.

## Acknowledgments

Funding: Supported by the Texas Commission on Environmental Quality (TCEQ) and the Environmental Protection Agency (EPA) National Aquatic Resource Survey's- National Rivers and Streams Assessment.

Additional support from the Environmental Institute of Houston.

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## Study Area

- Field work from May-September 2023-2024.
- 80 sites – Combination of revisit and random sample sites (Figure 1).

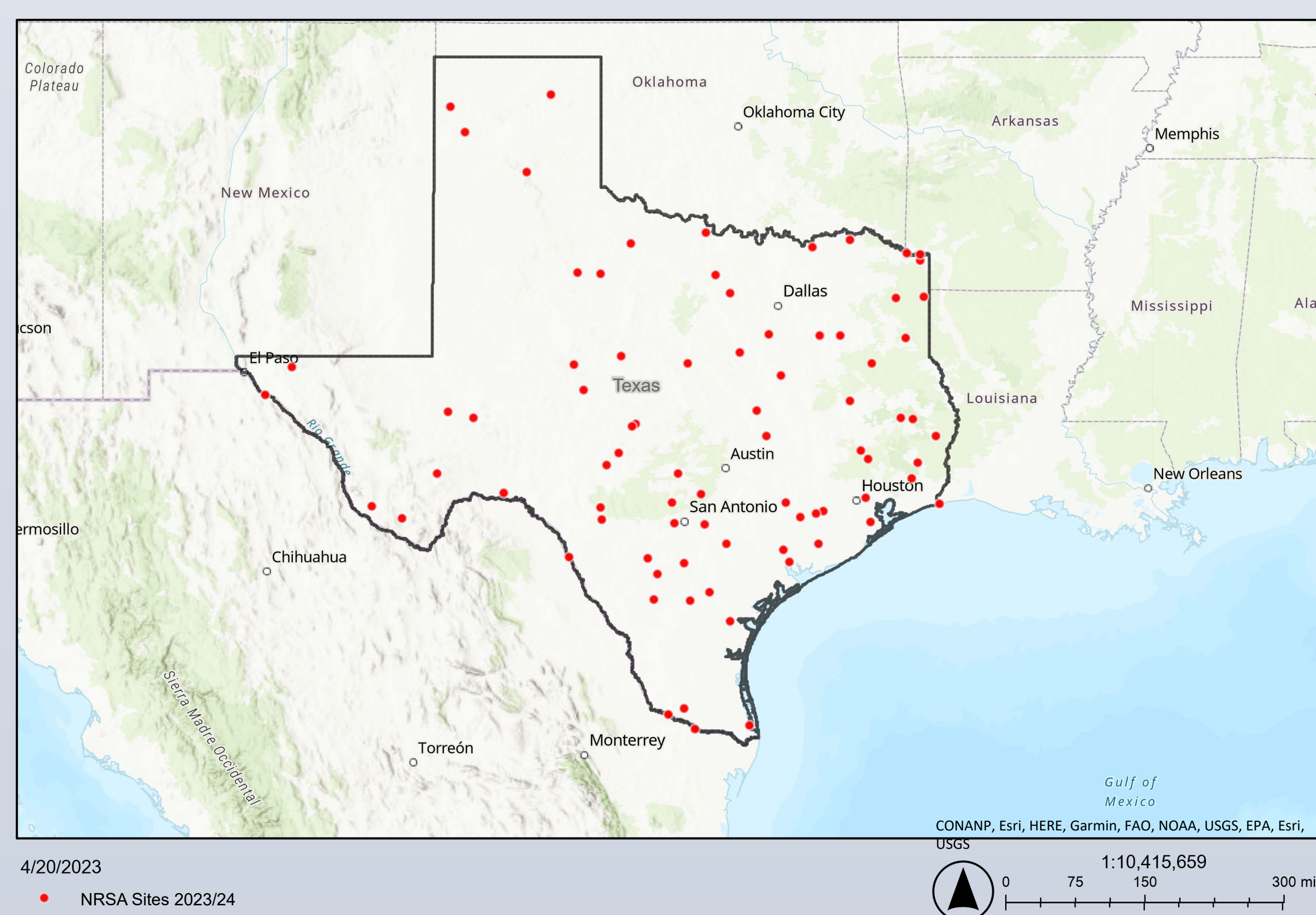


Figure 1: Map of the 80 base sites to be sampled during the 2023- 2024 sampling seasons across the state of Texas

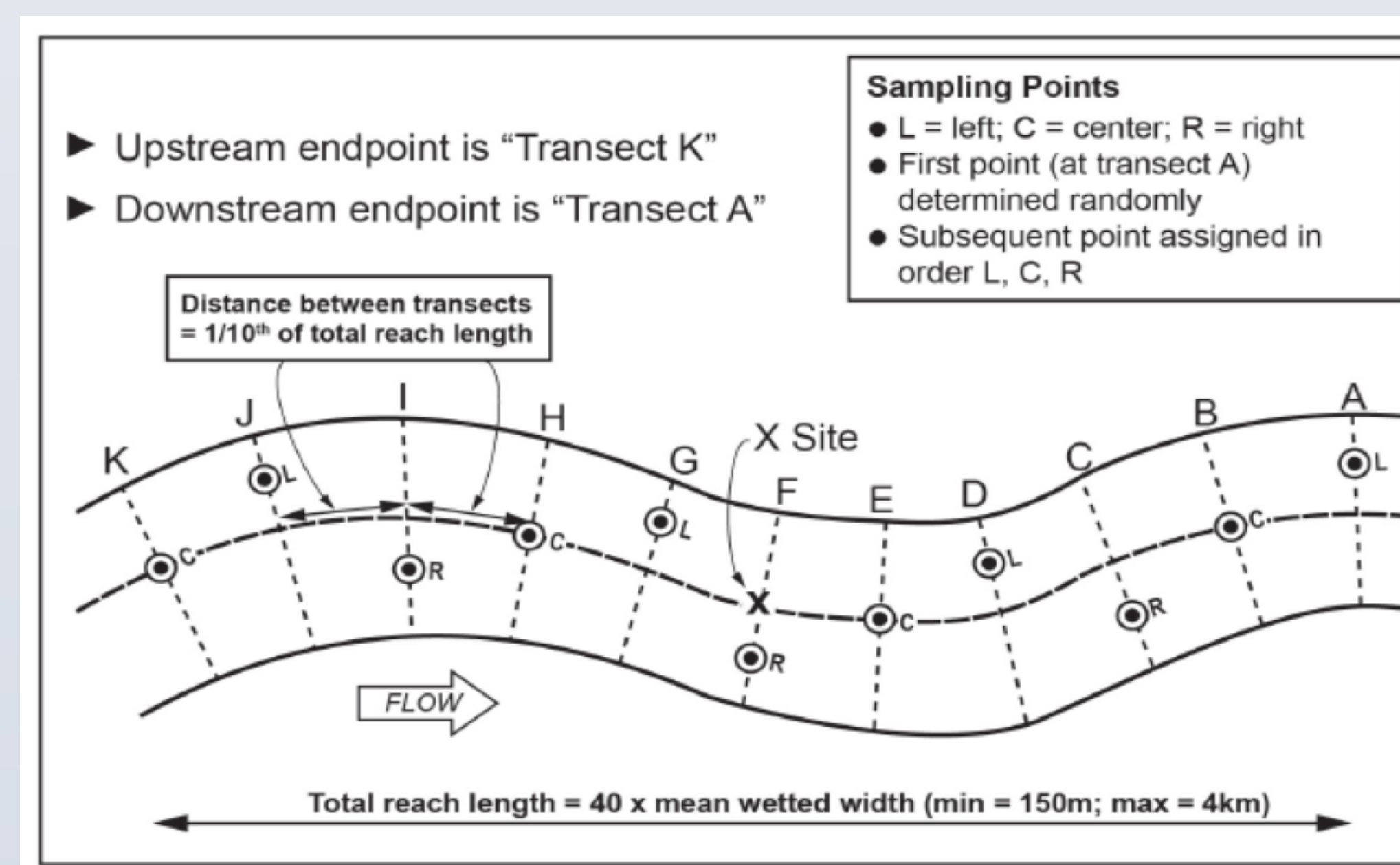


Figure 2: Wadeable: figure taken from NRSA Field Operations Manual (USEPA 2022a)

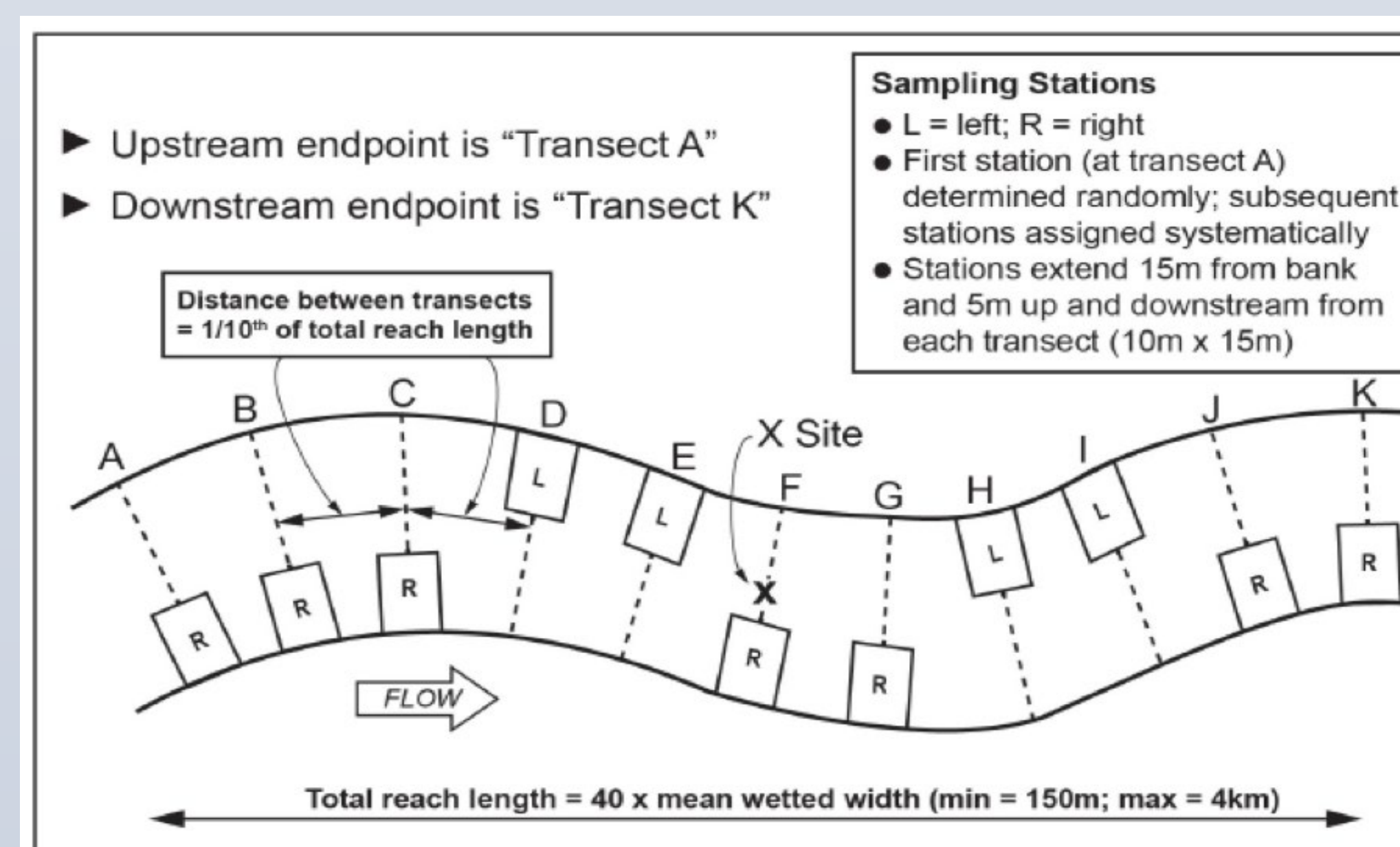


Figure 3: Non-wadeable: Figure taken from NRSA Field Operations Manual (USEPA 2022b)