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# Strong Interpopulation Divergence and Low Effective Population Sizes in Texas Alligator Snapping Turtles (*Macrochelys temminckii*)

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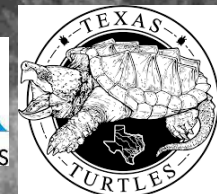
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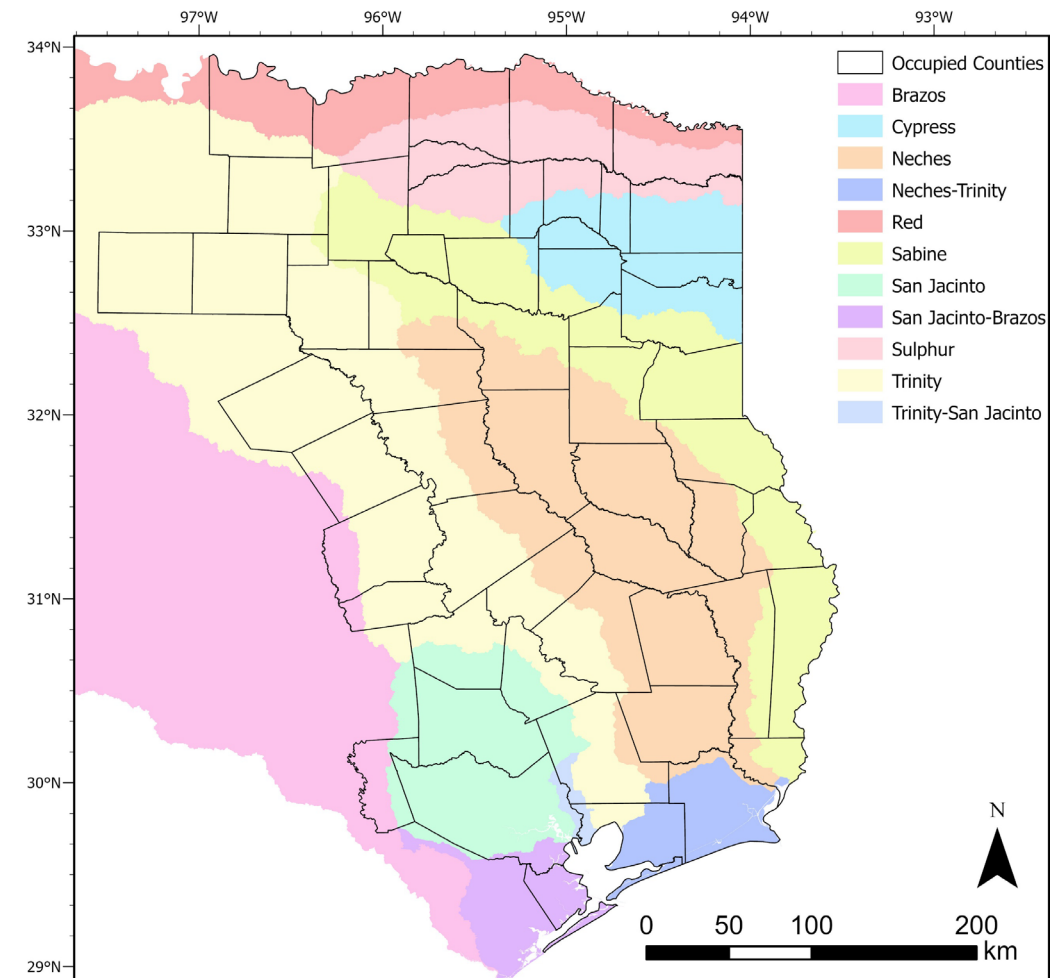


Texas Comptroller of Public Accounts



# Conservation Need

- Threatened status in Texas since 1987<sup>1</sup>
- Recent SSA recommends threatened status<sup>2</sup>
- Texas represents SW range edge<sup>3-4</sup>
- Range edges hypothesized to exhibit decreased genetic diversity<sup>5</sup>
- Documented range contractions at N and W range edges<sup>6-9</sup>
- Reintroduction already a conservation strategy in parts of range<sup>10-18</sup>



<sup>1</sup>Texas Register 1987, <sup>2</sup>USFWS 2021, <sup>3</sup>Dixon 2013, <sup>4</sup>Hibbitts and Hibbitts 2016, <sup>5</sup>Sexton et al. 2009, <sup>6</sup>Reidle et al. 2008, <sup>7</sup>Bluett et al. 2011, <sup>8</sup>Baxley et al. 2014, <sup>9</sup>Kessler et al. 2017, <sup>10</sup>Moore et al. 2013, <sup>11</sup>Anthony et al. 2015, <sup>12</sup>Townsend 2016, <sup>13</sup>Dreslik et al. 2017, <sup>14</sup>Glorioso et al. 2020, <sup>15</sup>Garig et al. 2021, <sup>16</sup>Hyder et al. 2021, <sup>17</sup>Cozad et al. 2023, <sup>18</sup>Voves et al. 2023

# Importance of regional patterns to population structure

- Historically strong genetic structure<sup>19-21</sup>
- With strong divergence, recommend maintaining divergence<sup>21-22</sup>
- Range-wide studies may miss regional patterns, especially with strong divergence<sup>22</sup>
- Regional patterns may be important in anthropogenically altered watersheds (e.g., dams)<sup>23</sup>
- 41 large dams in occupied Texas watersheds<sup>24-25</sup>
- Understanding how (or if) dams impact gene flow may help elucidate regional patterns



TPWD SPR-0212-019

<sup>19</sup>Roman et al. 1999, <sup>20</sup>Eschelle et al. 2010, <sup>21</sup>Apodaca et al. 2023, <sup>22</sup>Frankham et al. 2011,

<sup>23</sup>Bárcenas-García et al. 2022, <sup>24</sup>Texas Register 2009, <sup>25</sup>TCEQ 2021



# Study Objectives

1. Quantify existing population structure in the southwestern range edge
2. Measure genetic diversity within the existing population
3. Evaluate the potential effects of dams on genetic diversity





# Field Methods and Sample Collection

- Population assessments at varying scales across east Texas watersheds (USGS 8-digit HUC)<sup>26-32</sup>
- Baited hoop traps (91.4–121.9 cm diameter; 182.9–243.8 cm length)
- Sex and midline straight carapace length (male, female, juvenile; mid-SCL)
- Biopsy: webbing between posterior left digits 4 & 5 (5-mm punch)
- Whole blood: caudal sinus or dorsal coccygeal vein (0.5 to 3.0-cc; 21–25 gauge, 19.05–38.10 mm length needle)
  - Sodium heparin anticoagulant
  - Stored on wet ice; frozen within 6-12
  - Longmire's solution (3.03 g TRIS, 9.31 g EDTA, 2.5 g SDS, 250 mL water)



<sup>26</sup>Munscher et al. 2020, <sup>27</sup>Gordon et al. 2023a, <sup>28</sup>Gordon et al. 2023b, <sup>29</sup>Rosenbaum et al. 2023a, <sup>30</sup>Rosenbaum et al. 2023, <sup>31</sup>Munscher et al. 2023, <sup>32</sup>Ricardez et al. unpublished data

# Sequencing and Data Analyses

- Extractions using Quiagen Dneasy Blood & Tissue kit (protocol for muscle, blood, and skin)
- Constructed 3RAD libraries and barcoded samples<sup>33-34</sup> (i5 and i7 iTrue adapters)
- Removed individuals with < 1,000,000 raw reads and < 2,000 loci
- Identified single nucleotide polymorphisms (SNPs)
- Visualized and quantified population genetic structure using PCA (*ggplot2*) and fastSTRUCTURE<sup>35-36</sup>
- Quantified genetic diversity and population subdivision using *hierfstat* and NeEstimator<sup>37-41</sup>
  - Observed heterozygosity ( $H_O$ ), expected heterozygosity ( $H_S$ ), within-population subdivision ( $F_{IS}$ ), population connectedness ( $F_{ST}$ ), effective population size ( $N_e$ )
- Sub-set  $F_{ST}$  data for up- and downstream comparisons in relation to dams

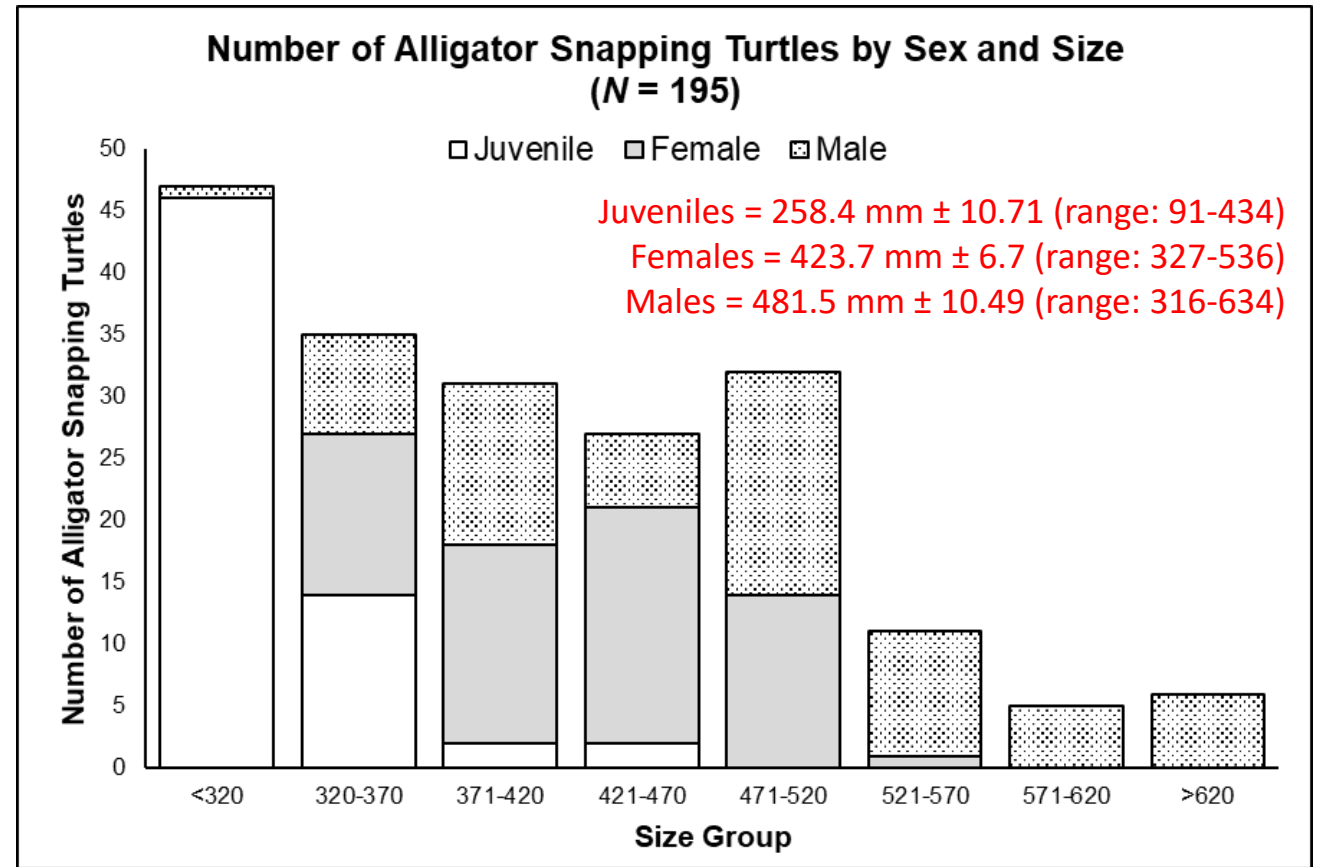
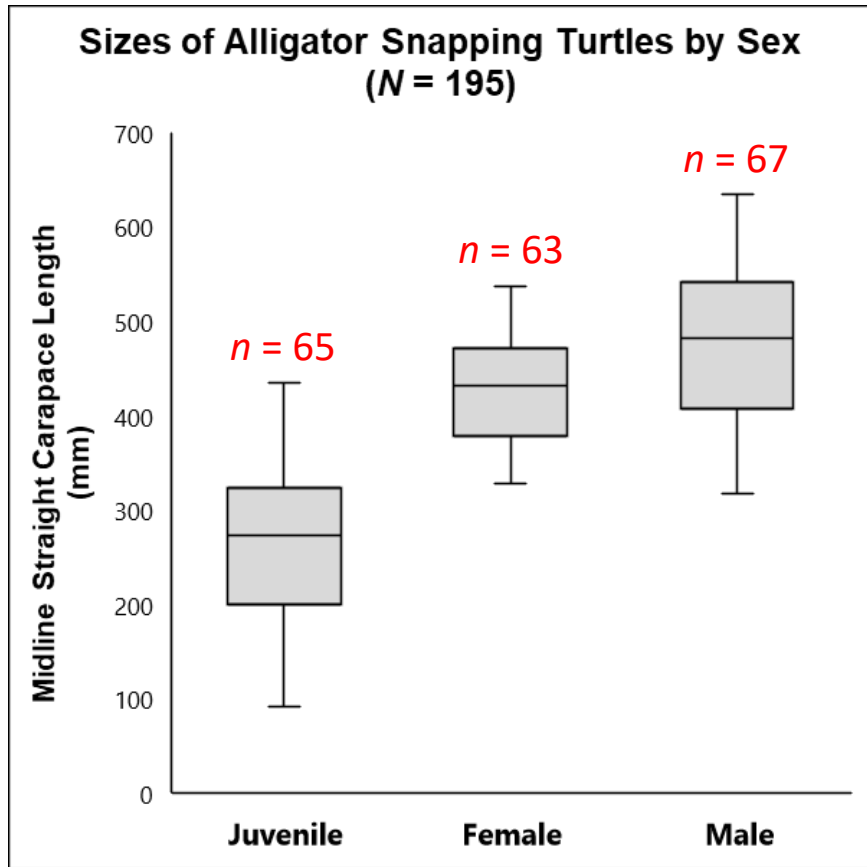
<sup>33</sup>Bayona-Vásquez et al. 2019, <sup>34</sup>Glenn et al. 2019, <sup>35</sup>Wickham and Change 2016, <sup>36</sup>Raj et al. 2014,

<sup>37</sup>Nei 1987, <sup>38</sup>Goudet 2005, <sup>39</sup>Weir and Goudet 2017, <sup>40</sup>Do et al. 2014, <sup>41</sup>Waples and Do 2010



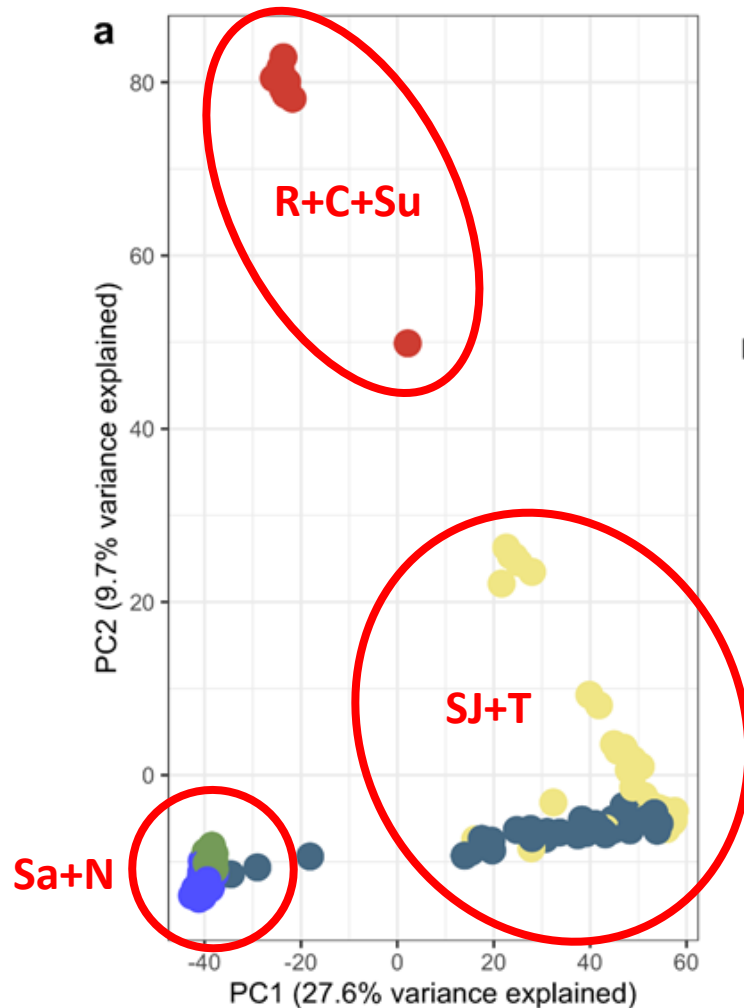
# Age and Size Class Distribution

- Total # individuals analyzed: 215
- Sex and morphometrics known for 195 individuals



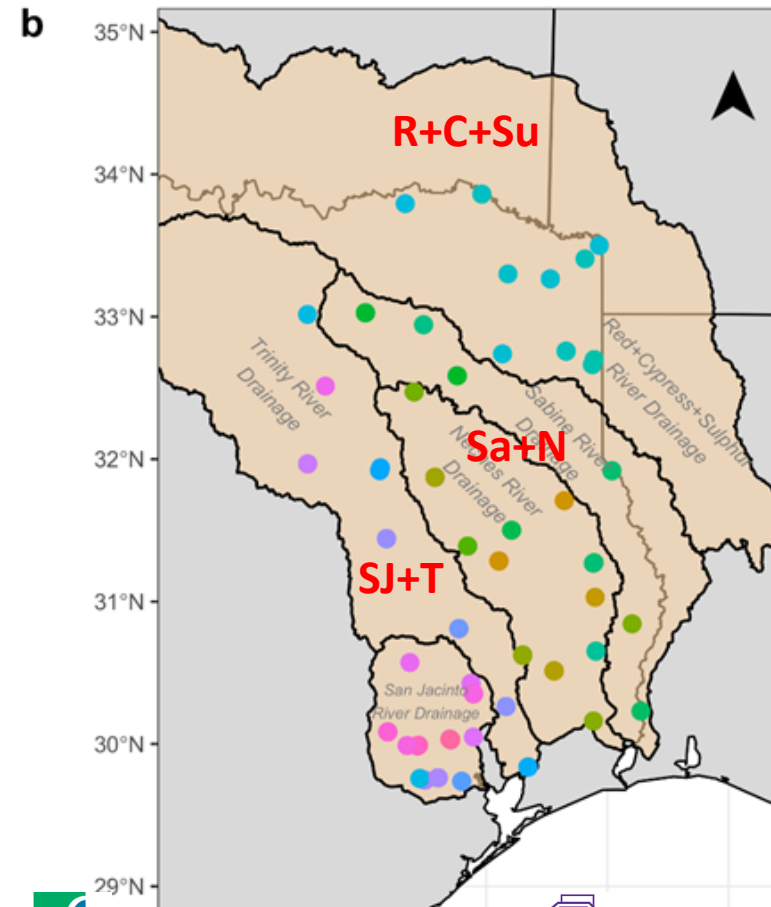
# Genomic Structure – Principal Components Analysis

- Full dataset = 571,259 unfiltered SNPs on 196,109 RAD loci
- PCA of 45,440 SNPs showed 3 major groupings (e.g., metapopulations)



#### Drainage

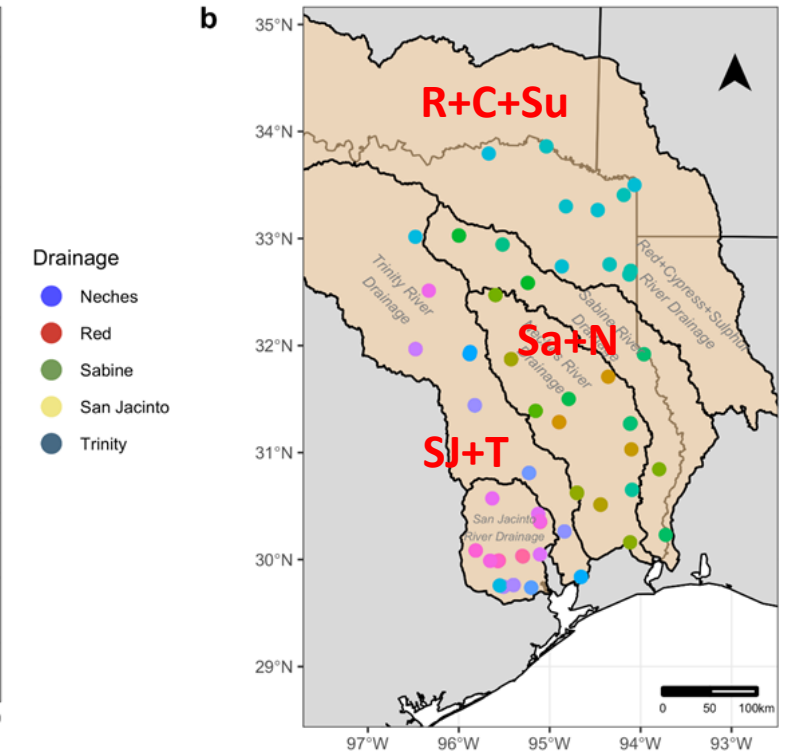
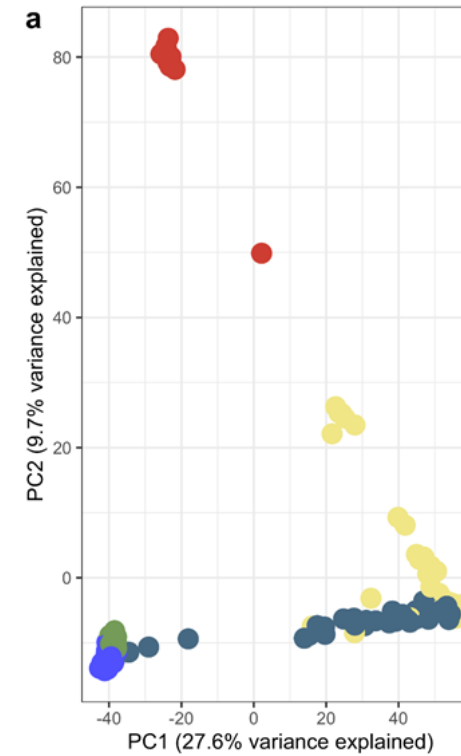
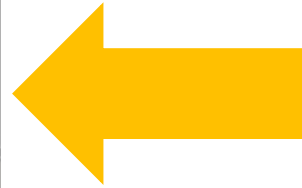
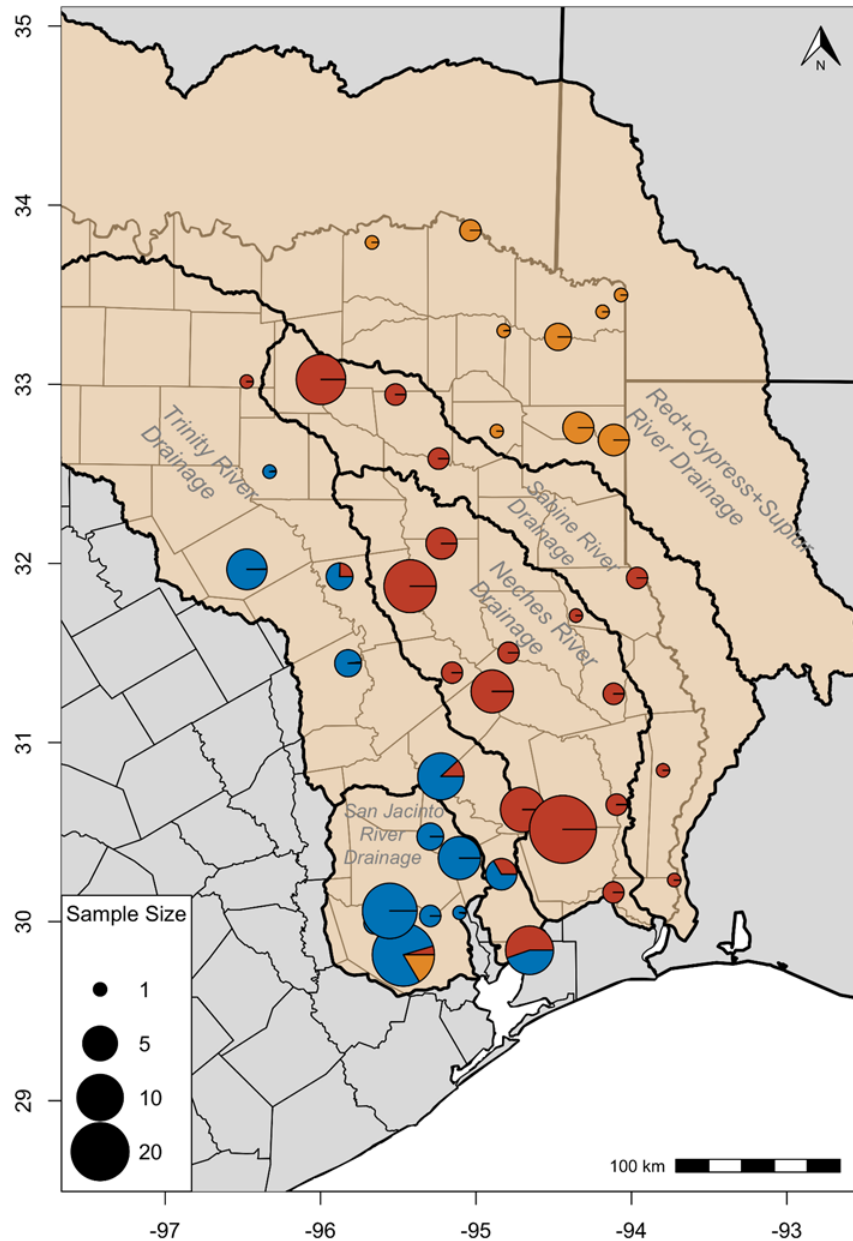
- Neches
- Red
- Sabine
- San Jacinto
- Trinity





# Genomic Structure – fastSTRUCTURE Analysis

- fastSTRUCTURE analyses on 30,064 SNPs confirmed three metapopulations (K = 3)



# Diversity and Population Subdivision

**Table 1** Sample size ( $n$ ), observed heterozygosity ( $H_o$ ; Nei 1987), within-population gene diversity (sometimes referred to as expected heterozygosity,  $H_s$ ; Nei 1987), within-population subdivision ( $F_{IS}$ ; Nei 1987), and effective population size ( $N_e$ ; Waples 2022) of Alligator Snapping Turtles (AST; *Macrochelys temminckii*) in Texas. Statistics are calculated for the three metapopulations determined by fastSTRUCTURE analysis (Red+Cypress+Sulphur, Sabine+Neches, and Trinity+San Jacinto).

Population	$n$	$H_o$	$H_s$	$F_{IS}$	$N_e$
Sabine+Neches (Sa+N)	97	0.0873	0.0904	0.0339	174.5 (117.6 - 311.2)
San Jacinto+Trinity (SJ+T)	100	0.0777	0.0864	0.1002	25.9 (25.9 - 25.9)
Red+Cypress+Sulphur (R+C+Su)	16	0.0696	0.0698	0.0038	444.4 (411.7 - 482.7)

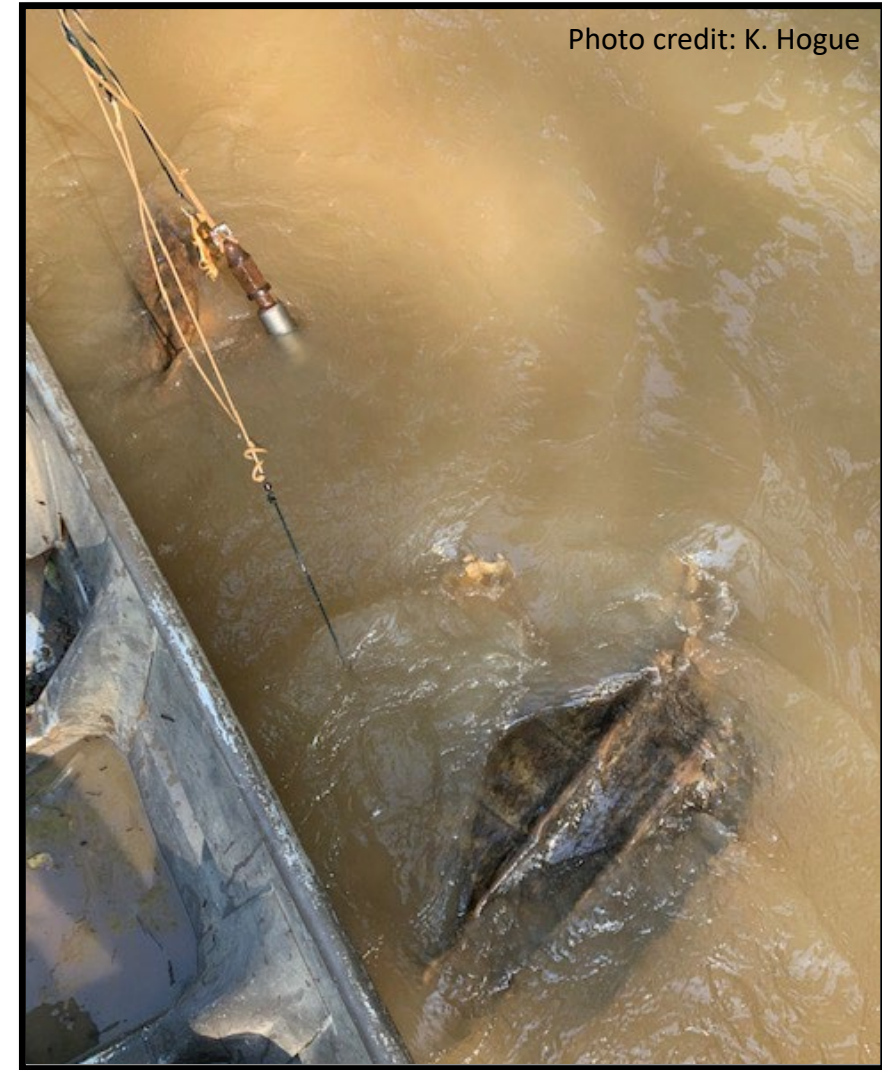
**Table 2** Population subdivision ( $F_{ST}$ ) for Alligator Snapping Turtle (AST; *Macrochelys temminckii*) populations in Texas as identified by fastSTRUCTURE analyses. See Table 1 for sample sizes.

Population $F_{ST}$ values	Red+Cypress+Sulphur (R+C+Su)	Sabine+Neches (Sa+N)	San Jacinto+Trinity (SJ+T)
Red+Cypress+Sulphur (R+C+Su)	NA	0.3885	0.4528
Sabine+Neches (Sa+N)	0.3885	NA	0.3110
San Jacinto+Trinity (SJ+T)	0.4528	0.3110	NA



# Potential Effect of Dams

- Permutation tests from upstream and downstream sites for:
  - Lake Livingston ( $n = 10$  up;  $n = 4$  down)
  - Richland-Chambers ( $n = 7$  up;  $n = 3$  down)
  - Blackburn Crossing ( $n = 3$  up;  $n = 14$  down)
  - Iron Bridge ( $n = 12$  up;  $n = 2$  down)
- Did not reveal any significant impact
- Overall  $F_{ST}$  was low
  - Lake Livingston  $F_{ST} = 0.002$  ( $p = 0.36$ )
  - Richland-Chambers  $F_{ST} = 0.027$  ( $p = 0.10$ )
  - Blackburn Crossing  $F_{ST} = 0.023$  ( $p = 0.06$ )
  - Iron Bridge  $F_{ST} = 0.000$  ( $p = 0.95$ )



# Conclusions

1. Quantify existing population structure in the southwestern range edge
  - $K = 3$  distinct metapopulations in Texas watersheds
  - Some admixture between metapopulations
2. Measure genetic diversity within the existing population
  - $H_O$ ,  $H_S$ ,  $F_{IS}$  all low (0.0696-0.1002 across all values)
  - $F_{ST}$  between metapopulations 0.311-0.452
  - $N_e < 100$  for at least one metapopulation (SJ+T)
3. Evaluate the potential effects of dams on genetic diversity
  - Results inconclusive
  - Artifact of long generation time and need for potentially 1,000s of years for detection





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Questions?

