**TECHNICAL PROJECT DESCRIPTION**

The primary objective of this study is to define the reference condition of macroinvertebrate assemblages in wadeable streams in the Northern Cross Timbers level IV ecoregion of central Oklahoma. The data will also be used to scale habitat and macroinvertebrate data collected by the Kickapoo Tribe of Oklahoma. Data from this study will be shared with other tribes and agencies engaged in water monitoring in the ecoregion.

The spatial scale of the study is the Northern Cross Timbers ecoregion; the time scale of the study is two years. Basic water quality data (water temperature, pH, specific conductance, turbidity, dissolved oxygen, total phosphorus, nitrate/nitrate and *E. coli*) will be collected eight times at each site during the two-year project period. Macroinvertebrates will be collected annually during the Summer Index Period (July 1-September 15) (OWRB, 2006). Water quality, habitat and macroinvertebrate data will be collected in accordance with our EPA-approved *Quality Assurance Project Plan for KDEP’s Surface Water Monitoring Program* (KDEP, 2015). The streams in the Northern Cross Timbers ecoregion are typically shallow and have sandy substrates (Woods et al., 2005). Sandy substrates support low macroinvertebrate diversity (Rinella and Feminella, 2005; Phillips, 2003). As a result, macroinvertebrates will be collected from aquatic vegetation and woody debris at each site.

“Reference condition” is used in the literature to mean a variety of conditions (Stoddard, Larsen, Hawkins, Johnson and Norris, 2006). All streams in the Northern Cross Timbers ecoregion are negatively impacted by human activity. As a result, in the context of this study “reference condition” means the **least disturbed condition** of wadable streams in the Northern Cross Timbers ecoregion, and will consist of a range of conditions, rather than a single state.

In the Reference Condition Approach (RCA) (Bailey, Norris and Reynoldson, 2004), the site is the sampling unit and the goal of a RCA study is to characterize variation between sites in reference condition. It is, therefore, not helpful to collect multiple samples at one site because the samples will almost certainly be correlated with one another. For the purpose of this study, sites must be separated by a minimum of 100 m. It is preferably that only one site be located on each stream, but this may not be possible on higher order streams (Bailey et al, 2004).

When applying the Reference Condition Approach, it is essential that the criteria for selecting reference sites are established *a priori* (Bailey et al., 2004). The structure of the macroinvertebrate community is not taken into account when defining reference criteria because (1) the purpose of the study is to define reference conditions and then assess typical biota, so including biota in the criteria for selecting reference sites creates a circular argument (Bailey et al, 2004) and (2) we want to capture the natural variability among reference sites and do not know *a priori* how much variation is typical in the least disturbed macroinvertebrate assemblages in the Northern Cross Timbers ecoregion (Stoddard et al., 2006). The criteria for selecting reference condition sites for this study are as follows:

1) The stream segment is not on the 303(d) list.

2) The stream segment is not subjected to significant regulation of flow.

3) The stream is free of point source discharges.

4) The site is more than 50 m upstream or 300 m downstream from a significant

confluence

5) The stream segment has an intact riparian area (>30 m from each bank).

6) The land use of the catchment is < 20% urbanized.

Reference sites will be selected based on geospatial data. Possible data sets for the selection of reference condition sites include NPDES point source discharges, aerial photographs, and land use and land cover data. Reference sites will be randomly selected from the total population of sites that meet the reference condition criteria to enable later use of inferential statistics. Sites may be removed from the reference condition sample if unexpected impairments or instream habitats that are not represented in the test sites are discovered during the initial or subsequent visits. They will not be removed, however, solely on the basis of an impoverished macroinvertebrate fauna.

According to Bailey et al. (2004), 25 reference condition sites is the minimum number necessary for a pilot project. If there is little variation among reference sites, 25 reference sites may be sufficient to assess test sites (Bailey et al., 2004). Although 25 reference condition sites constitute a less than ideal sample size, fewer test sites are needed in study areas with less spatial complexity (Bailey et al., 2004).

The data analysis for this study will include calculating means, ranges and standard deviations for the following parameters:

Catchment characteristics:

1. Catchment area

2. Percent of catchment with an urbanized land use

3. Percent of catchment with an agricultural land use

Rapid Bioassessment Protocols Habitat Parameters:

4. Epifaunal Substrate/Available Cover score

5. Pool Substrate Characterization score

6. Pool Variability score

7. Sediment Deposition score

8. Channel Flow Status score

9. Channel Alteration score

10. Channel Sinuosity score

11. Bank Stability (right and left bank averaged for each site) score

12. Vegetative Protection (right and left bank averaged for each site) score

13. Riparian Vegetative Zone Width (right and left bank averaged for each site) score

Macroinvertebrate measures:

14. Total macroinvertebrate species richness, identified to genus/species

15. Total EPT richness, identified to genus/species

16. Total intolerant taxa richness genus/species

17. Shannon-Wiener H’ (log2)

18. A modified Hilsenhoff Biotic Index (HBI)

Calculation of descriptive statistics for each of these parameters will give us an idea of the normal range of catchment, habitat, and macroinvertebrate measures for reference sites in the Northern Cross Timbers Region. It will also allow us to begin exploring which environmental variables might contribute most to increases or decreases in macroinvertebrate measures. Percent of catchment with an urbanized or agricultural land use will be calculated, but should not be used for later model building because predictor variables used in model building must be unrelated to stressors likely to occur in the study area (Bailey et al., 2004). Taxa richness, EPT richness, Shannon diversity, and a modified HBI demonstrated consistent separation between “good” and “poor” sites in a meta-analysis of macroinvertebrate data collected by the Oklahoma Conservation Commission (OCC, 2005).

Ideally, the data from this study would be used to construct a predictive model using ordination, multiple regression, or discriminate function analysis (DFA). If catchment and habitat measures explained a sufficient proportion of the variation in macroinvertebrate measures at sites in reference condition, we could then use the model to determine whether a test site was in reference condition. We do not currently have the internal capacity to construct a predictive model. However, if this study is funded, we will seek out technical and financial assistance to complete further statistical analysis and model building using the collected data.