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STATUS SURVEY OF EIGHT RARE AQUATIC MACROINVERTEBRATES IN EAST TEXAS

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STATUS SURVEY OF EIGHT RARE AQUATIC MACROINVERTEBRATES IN EAST TEXAS

by

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STATUS SURVEY OF EIGHT RARE AQUATIC MACROINVERTEBRATES IN EAST TEXAS

Abstract

Freshwater is essential to human existence. The health of each freshwater source is connected to the survival of pollution intolerant species of aquatic macroinvertebrates in the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT taxa). A higher presence of EPT taxa equates to better water quality because their water bound larvae assist in maintaining clean freshwater environments and will not survive in highly polluted water. Human disruption to natural environments is causing increases in polluted freshwater, leading to decreases in the presence of EPT taxa. Texas Parks and Wildlife Department documentation shows major concern for the populations of eight EPT species of critical concern in East Texas (*Sparbarus coushatta, Tricorythodes curvatus, Isoperla sagittata, Cheumatopsyche morsei, Chimarra holzenthali, Hydroptila ouachita, Neotrichia mobilensis, and Phylocentropus harrisi).* A field status survey of each species was based on an extensive literature review and included their documented historical locations. Evidence of *S. coushatta*, *T. curvatus* and *I. sagittata* was found at and near their historical locations. There was no evidence for the presence of *C. morsei, C. holzenthali, H. ouachita, N. mobilensis*, or *P. harrisi.* Canonical correspondence analysis showed the significance of species findings in comparison to each other, their habitats, and water quality of each sample location. Man-made threats to Texas aquatic macroinvertebrate habitats have increased exponentially and leave these species with a status of greatest conservation need.

CHAPTER 1

Introduction

Sources of freshwater for humans include surface water and groundwater (Dieter & Maupin, 2017). Approximately 70% of human freshwater usage comes from surface water in the form of streams, rivers, and lakes (Dieter & Maupin, 2017). Each of these surface water sources provides sanctuary to an abundance of aquatic macroinvertebrate species (Jackson & Fuereder, 2006). Because of human disruption and pollution of freshwater habitats, many aquatic macroinvertebrate populations are in decline (Jackson & Fuereder, 2006; Ab Hamid & Rawi, 2017). In Texas, billions of gallons of freshwater are used for human health, cooling power plants, agriculture irrigation, industrial irrigation, construction, and recreation (Parker et al., 2000; Stillwell et al., 2011; Cabrera et al., 2013). While the ability to use billions of gallons of water at will is enticing, freshwater sources are not unlimited and understanding how humans affect sources is important to conserving them. Although aquatic macroinvertebrates may seem insignificant, they serve as an essential tool for humans to assess freshwater quality (Jackson & Fuereder, 2006). High freshwater quality aligns with an increased presence of aquatic macroinvertebrate species from three specific orders: Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies; EPT taxa) (Ab Hamid & Rawi, 2017). Higher diversity of EPT taxa indicates a healthier freshwater system because species in these orders are pollution intolerant (Brittain, 1990; Barber-James et al., 2007; Rasmussen et al., 2008). If no EPT taxa are found, the water is very low quality (Jackson & Fuereder, 2006; Ab Hamid & Rawi, 2017). This method of determining freshwater quality is also known as the EPT Taxa Richness Index (Ab Hamid & Rawi, 2017).

Aside from the benefit of determining water health for human use, EPT taxa also play an important role in improving environmental health for terrestrial and aquatic ecosystems. Mayflies, stoneflies, and caddisflies begin life in aquatic larval stages, but metamorphosize into terrestrial winged adults (Ab Hamid & Rawi, 2017). At the larval stage, herbivorous EPT taxa contribute to the ecosystem by helping breakdown organic matter in the water column, such as algae, bacteria, debris, and dead or decaying particulates (Brittain, 1982; Brittain, 1990; Wiggins, 2004; Ab Hamid & Rawi, 2017). The decomposition assistance provided by EPT taxa is essential to maintain the balance of nutrient cycling in aquatic ecosystems (Brittain, 1982; Wiggins, 2004; Wesner et al., 2017). Carnivorous and omnivorous EPT taxa larvae are beneficial for the terrestrial world because they feed on pest fly eggs and larvae, reducing the populations of biting midges and mosquitos (Brittain, 1982). In aquatic and terrestrial food webs, EPT taxa also serve as a significant source of nutrition for several small predators, including amphibians, fish, larger macroinvertebrates, mammals, reptiles, birds, and arachnids (Wesner et al., 2017). Mayflies, stoneflies, and caddisflies may be small, but they are crucial for wellbeing of aquatic and terrestrial environments.

Our eight species of interest for this status survey are all EPT taxa. The Ephemeroptera (mayfly) species of interest are *Sparbarus coushatta* and *Tricorythodes curvatus.* The Plecoptera (stonefly) species of interest is *Isoperla sagittata.* The Trichoptera (caddisfly) species of interest are *Cheumatopsyche morsei, Chimarra holzenthali, Hydroptila ouachita, Neotrichia mobilensis* and *Phylocentropus harrisi.*

Objectives

The objectives for this project are: (1) Determine the current distribution of eight rare aquatic macroinvertebrate species larvae across their geographic range in East Texas, and (2)

Determine habitat use, threats, and conservation status of the eight species. Populations of the eight target species are currently considered critical concern and extremely rare in East Texas (Texas Parks & Wildlife, 2020). Completion of this status survey will increase our knowledge of each species' viability or risk of extirpation.

Hypothesis – The eight species will be found at more pristine sites with higher water quality.

Chapter 2

Literature Review

An extensive literature review uncovered historical locations, surface water preferences, and information about larvae identification for each target species. Nine East Texas counties harbored historical sites for the eight EPT species of interest: Anderson, Austin, Hardin, Johnson, Montgomery, Newton, Polk, San Jacinto, and Tyler (Fig. 1; Szczytko & Stewart, 1976; Moulton & Stewart, 1993; Abbott et al., 1997; Baumgardner & Wiersema, 1999; Sun & McCafferty, 2008).

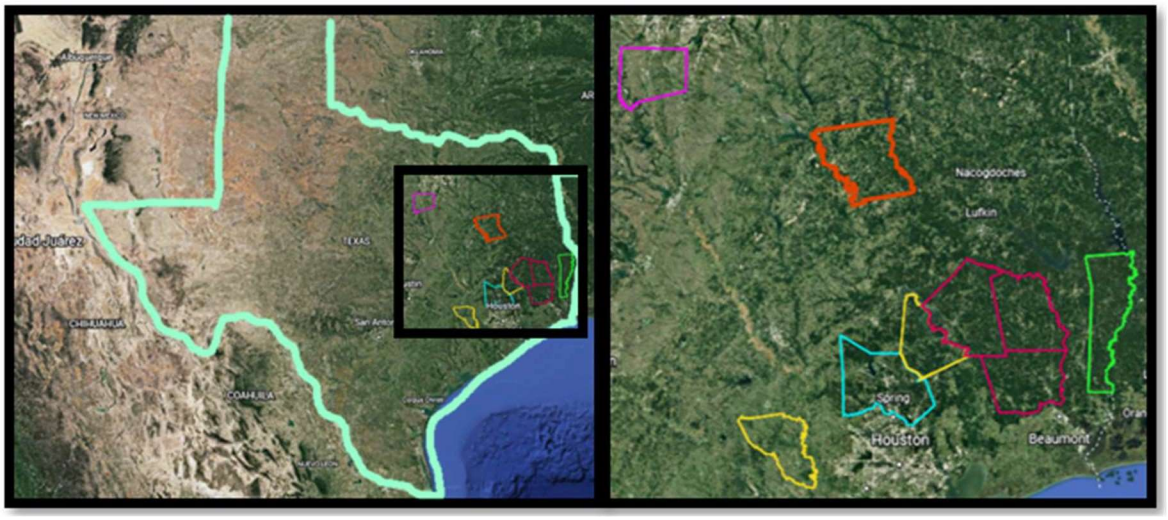

Figure 1. Summary of all historical county sites in East Texas for *S. coushatta* (yellow = Austin and San Jacinto Counties), *T. curvatus* (blue = Montgomery County), *I. sagittata* (green = Newton County), *C. morsei, C. holzenthali, H. ouachita* (orange = Anderson County), *N. mobilensis* (pink = Johnson County), *and P. harrisi* (red = Hardin, Polk, and Tyler Counties). County map was created using Google Earth.

Ephemeroptera

Sparbarus coushatta was formally identified as a species in 2008 from larvae sampled in 1997 from Austin County and 1998 from San Jacinto County, Texas (Fig. 1; Sun & McCafferty, 2008). One larva was identified from a San Bernard River sample in Austin County (Sun & McCafferty, 2008). One larva was identified from a Winter's Bayou sample and four larvae from San Jacinto River samples in San Jacinto County (Sun & McCafferty, 2008). Based on previous records, this species has only been found in East Texas.

Historical identification of *S. coushatta* larvae was determined from the following observations (Sun & McCafferty, 2008):

- The dorsal abdomen often has a black mark across the left side. This mark is sometimes absent.
- Abdominal dorsal plates 7, 8, and 9 often have a centered, lengthwise mark.
- The second segment of the head antennae are two and half times the length of the first segment.
- There is a distinction of segment size comparisons for the labrum, hypopharynx, labial palp, and maxilla.
- The dorsal head has a triangular middle ocellar tubercle and two crescent side ocellar tubercles.
- The dorsal head aligns with the connection to the abdominal segment.
- The body has asymmetric, speckled black markings.

Tricorythodes curvatus was formally identified as a species in 1977 from a mature larvae found in Independence County, Arkansas (Allen, 1977). Three larvae were identified from White

River samples in Independence County, Arkansas (Allen, 1977). Presence of *T. curvatus* in Crawford County, Missouri and Montgomery County, Texas was confirmed in 1999 (Fig 1; Baumgardner & Wiersema, 1999; Nichols & Sites, 1999). Hundreds of adults and larvae were identified from Meramec River samples in Crawford County, Missouri (Nichols & Sites, 1999). Twenty larvae were identified from Caney Creek samples in Montgomery County, Texas (Fig 1; Baumgardner & Wiersema, 1999).

Historical identification of *T. curvatus* larvae was determined from the following observations (Allen, 1977):

- Males and females are difficult to differentiate because they share the same physical features.
- Males have a distinctly smaller eye size in comparison to males of similar species.
- The posterior of the maxilla does not form a primary denticle.
- Black coloration is stretched throughout the operculate gill.

Plecoptera

Isoperla sagittata was formally identified as a species in 1976 from adults and larvae sampled from Newton County, Texas (Fig 1; Szczytko & Stewart, 1977). Five adults and 12 larvae were identified from Little Cow Creek samples (Szcytko & Stewart, 1977). Based on these records, this species has only been found in East Texas.

Historical identification of *I. sagittata* larvae was determined from the following observations (Szcytko & Stewart, 1977):

- Males have reduced lobes following the tenth abdomen segment and two protruding lobes near the dorsal terminus.

- Females form a downward slanted plate over their genitals.
- The posterior of the maxilla generates hairs below the primary denticle.
- Larvae have distinct mouthparts: upper mouthpart rectangular with a central protrusion, setae below subapical tooth, split between the glossae, and unique mandible teeth ratios.

Trichoptera

Cheumatopsyche morsei was formally identified as a species in 1974 from adults found in Jackson Parish, Louisiana (Gordon, 1974). Six adults were identified from Schoolhouse Spring samples (Gordon, 1974). Based on one record, *C. morsei* may also be found in East Texas (Moulton & Stewart, 1997).

Historical identification of *C. morsei* larvae was determined from the following genitalia sclerotization observations (Gordon, 1974):

- The extension and angle of the phallotheca is distinct from all other Cheumatopsyche species.
- Female genitalia have clasper matching the length of a narrow 10^{th} terga.
- Dorsal sclerites are present on the female genitalia's median plate.

Chimarra holzenthali was formally identified as a species in 1987 from adults found in Jackson Parish, Louisiana (Lago & Harris, 1987). Seven adults were identified from Schoolhouse Spring samples (Lago & Harris, 1987). Presence of *C. holzenthali* in Anderson County, Texas was published in 1993 (Bowles et al., 1993). One adult was identified from Salmon, Texas (Bowles et al., 1993).

Historical identification of *C. holzenthali* larvae was determined from the following genitalia sclerotization observations (Lago & Harris, 1987):

- The elongate preanal limbs and the sclerotized ridge of the middle male genitalia limbs have a unique shape.
- Caudal face of the inferior male genitalia appendage resembles a quarry.
- Male genitalia have a minor, sclerotized lobe beneath the dorsal spur.

Hydroptila ouachita was formally identified as a species in 1983 from larvae found in Jackson Parish, Louisiana (Holzenthal & Kelley, 1983). Twelve larvae were identified from Schoolhouse Spring samples (Hozenthal & Kelley, 1983). Based on one record, *H. ouachita* may also be found in East Texas (Moulton & Stewart, 1997).

Historical identification of *H. ouachita* larvae was determined from the following genitalia sclerotization observations (Holzenthal & Kelley, 1983):

- Male genitalia have sword like middle limbs.
- Distinct presence of three dark, hardened marks on the primary layer of the inferior limbs of the male genitalia.

Neotrichia mobilensis was formally identified as a species in 1985 from adults found in Mobile County, Alabama (Harris, 1985). *N. mobilensis* was found in great abundance in three Alabama counties: Baldwin, Mobile, and Washington (Harris & Rasmussen, 2010). In Baldwin County, 20 adults were identified from Kettle Creek samples; 316 adults from Baldwin Creek Tributary; 21 adults from Tensaw Lake Slough; 16 adults from Proctor Creek; eight adults from Red Hills Creek; four adults from Squirrel Bayou; 28 larvae from North Rice Creek; 49 adults

from Bayou Tallapoosa; and 144 adults from the Tensaw Lake inlet (Harris & Rasmussen, 2010). In Mobile County, Alabama, 19 adults were identified from Mobile River samples and three adults from the Dead Lake Marina (Harris, 1985; Harris & Rasmussen, 2010). In Washington County, Alabama, 45 adults were identified from the Tombigbee River (Harris & Rasmussen, 2010). Presence of *N. mobilensis* in Johnson County, Texas was published in 1993 (Fig. 1; Moulton et al., 1993). Adults were identified from Ham Creek samples (Moulton et al., 1993).

Historical identification of *N. mobilensis* larvae was determined from the following genitalia sclerotization and habitat observations (Harris & Rasmussen, 2010):

- Male genitalia have a single rather than double protrusion on the phallus.
- Female genitalia have no sternal plate covering over the abdomen and sometimes have a small protrusion.
- The copulation pouch on females has distinct proportions in relation to its chambers and sclerites.
- Larvae for *N. mobilensis* are found near riffles and fast-moving water.
- Mature larvae sewn into fine grain cases and attached to rocks exhibit formation of distinct genitalia.

Phylocentropus harrisi was formally identified as a species in 1984 from an adult found in Baldwin County, Alabama (Schuster & Hamilton, 1984). In Baldwin County, one adult was identified from a 1982 Pine Log Creek sample, one adult from a 2004 Tensaw Lake Slough sample, one adult from a 2004 Squirrel Bayou sample, and one adult from a 2004 Bayou Tallapoosa East sample (Harris, 1984; Schuster & Hamilton, 1984; O'Neil, 2004; O'Neil &

Moss, 2004). Presence of *P. harrisi* adults in Texas was published in 1997 with reference to the Big Thicket National Preserve Area of Hardin, Polk, and Tyler Counties (Abbott et al., 1997). No evidence of *P. harrisi* larvae have been recorded.

Historical identification of *P. harrisi* larvae has not been determined (Sturkie & Morse, 1998; Pescador et al., 2004):

- Of the five known *Phylocentropus* species, larvae of three species have been identified, including *P. carolinus, P. lucidus,* and *P. placidus* (Sturkie & Morse, 1998).

Museum Record Review

Based on the literature review, UT Tyler's Department of Biology contains aquatic macroinvertebrate museum records from two freshwater localities in East Texas that may serve as homes to our target species: the Neches River and Mud Creek. The Neches River runs through historical counties for *P. harrisi:* Tyler and Hardin. Mud Creek runs through a historical county for *P. harrisi*: Polk. Museum record reviews for the Neches River and Mud Creek included searching for larvae from the *P. harrisi* family Dipseudopsidae. Completion of the museum record review revealed no specimen matching the family identification of Dipseudopsidae.

CHAPTER 3

Methods

Based on historical locations, Texas Parks and Wildlife approved status survey sampling in the counties surrounding the historical sites. We determined sample sites using Google Earth, Texas Watershed Viewer, and National Park Service maps of the Big Thicket National Preserve in East Texas. Eight historical sites, one historical area, and 21 new sites with similar habitats were sampled for the EPT taxa species of interest for a total of 30 sample sites. Sampling was completed in seven days between spring and summer of 2023. Texas sampling timeline: *I. sagittata* – February 2023; *S. coushatta* and *T. curvatus* – June 2023; *C. morsei, C. holzenthali, H. ouachita,* and *N. mobilensis* – June 2023; *P. harrisi* – July 2023.

Field Collection

At each freshwater sampling site, areas with debris piles, rocks and loose sediment were evaluated as possible macroinvertebrate habitats. Rocks in the water were turned to check for clingy populations. Tweezers were used to carefully move macroinvertebrates clinging to the turned rocks to double layered Ziploc freezer bags. D-frame dip nets were placed at the base of the water downflow for individual net collections. Buried macroinvertebrates were whisked toward the D-frame dip nets by repeatedly digging a hand or foot into the base habitat. D-frame dip net collections were emptied into double layered Ziploc freezer bags. After individual site collections were completed, 95% ethanol was added to each sample bag for specimen preservation, along with labels of site name and sample date. Samples were transported back to the research lab at UT Tyler for processing.

Laboratory Rearing of Caddisflies

These collection methods were only utilized for targeting live samples of the five target caddisfly species: *C. morsei*, *C. holzenthali*, *H. ouachita*, *N. mobilensis*, and *P. harrisi*. Live samples were needed because identification of each caddisfly species is dependent on the sclerotization of their genitalia in mature larvae growth stages. At each caddisfly site, free-living and cased larvae from the target species families were identified, carefully picked and placed into a travel aquarium with an actively running bubble device to maintain proper oxygen. The live caddisfly samples were transported back to UT Tyler for processing.

Water Quality Tests

Water quality data were collected in the field using a Vernier LabQuest 3 data collection device and data-specific probe attachments. Temperature was measured in degrees Celsius. Dissolved oxygen levels were measured in parts per million (mg/L) to determine the amount of oxygen in the aquatic environment. The pH level was measured to determine if the water's acidity level is within normal limits. Conductivity was measured in micro-Siemens per meter (μS/m). Turbidity was measured in nephelometric turbidity units (NTU).

Habitat Assessments

Each site's aquatic and terrestrial habitats were assessed with journal and photographic documentation. Recorded habitat features include: riparian zone features, surface water type, sediment composition, flow patterns, presence of riffles or waterfalls, meandering, and vegetation presence in the water column.

Sample Processing for EtOH Collection

Aquatic macroinvertebrates were carefully picked out of the debris. Each site's sample bags were separated into small portions to be viewed in a dissection tray filled with water. Desk lamp lights were directed at the water to reveal small macroinvertebrates floating on the surface layer. Tweezers were used to carefully pick macroinvertebrates from the tray and place them into small glass vials. Vials at this processing stage were separated into four categories: mayfly larvae, stonefly larvae, caddisfly larvae, and mixed community. Labels for each vial included the sample site name, date of sample, and macroinvertebrate category. When no macroinvertebrates were found for 20 minutes, the tray sample was emptied and rinsed through three layered sifting screens. Remaining sample contents in the screens was rinsed back onto the tray and checked for uncovered macroinvertebrates. If no macroinvertebrates were found for 20 minutes, the contents of the tray were sifted to drain the water and the organic material was discarded. These steps were repeated for each portion of the sample bag in process. After sample bags were completely processed, macroinvertebrates in each vial were identified to the family level. If found, the following families in each vial were separated for identification to the species level: mayfly larvae vials – Caenidae (*S. coushatta*) and Leptohyphidae (*T. curvatus*); stonefly larvae vial – Perlodidae (*I. sagittata*); caddisfly larvae vials – Dipseudopsidae (*P. harrisi*), Hydropsychidae (*C. morsei*), Hydroptilidae (*H. ouachita and N. mobilensis*), and Philopotamidae (*C. holzenthali*). Families of target species were compared to the most similar species for identification.

Sample Processing for Live Collection

Rearing live caddisfly in an artificial setting was necessary to verify the larvae had grown to an appropriate maturity with genitalia sclerotization. This was a crucial step because caddisflies are only identifiable to the species level based on mature genitalia features. Live caddisfly larvae were separated based on target species families and sites, then placed into modified containers with netting to prevent emergent individuals from escaping the enclosures. The enclosures were designed to function as netted rearing enclosures. To contrast the appearance of the larvae in the enclosures, bright colored sediment and plants were used as the base environment. Each enclosure was connected to the Aquatic Habitats System (AHAB) by a single tube with continuous filtered water flow. A release tube on the anterior end of each enclosure assisted in maintaining a balanced level of water intake and outtake. Water was regularly checked and maintained to assure the water chemistry was sufficient for healthy caddisfly life. Live caddisfly specimens were viewed under a dissecting microscope to note physical changes at metamorphosis stages.

Figure 2. Aquatic Habitats System rearing enclosures.

Identification of Live Collection Species with KOH Saturation

As live caddisfly larvae reached a more mature stage with sclerotized genitalia, specimens were removed from their enclosure and saturated with potassium hydroxide (KOH) (Blahnik et al., 2007). This method has been utilized for several decades in caddisfly identification and is useful to make their bodies translucent to reveal genitalia structures (Blahnik et al., 2007). This method was successfully tested with non-target specimens of caddisfly larvae to ensure the KOH properly saturated the bodies for internal viewing. After maturity was reached for a target specimen, they were viewed under a compound microscope (Blahnik et al., 2007).

Statistical Methods

Fold Change

Fold change was calculated to find population fluctuations in sites that had a historical population (Table 13). Fold change = current population count divided by the historical population count (Buchwalter et al., 2015). Sites with fold change values above 1 had a (#)-fold increase. Sites with fold change values below 1 had a (#)-fold decrease. Sites with fold change values of 0 had no-fold change (Table 13).

Logistic Regression

Logistic regression results were calculated in Microsoft Excel using each site's water quality analysis and identified target species presence or absence values (Table 14). P-values <0.05 determined if there were significant relationships between variables (Visser et al., 2017; Aweng et al., 2022). Slope values determined if there was a negative or positive correlation between variables (Li et al., 2012).

Shannon's Diversity Index (H')

H' results were calculated in Microsoft Excel using aquatic macroinvertebrate community data from all sample sites (Tables 1, 3, & 5). H' = community diversity based on variation and abundance of each aquatic macroinvertebrate family; $H=-\sum[(pi)\times ln(pi)]$ (Godfrey, 1978; Lewis & Harrel, 1978; Türkmen & Kazanci, 2010). Higher values were the most significant (Godfrey, 1978; Lewis & Harrel, 1978; Türkmen & Kazanci, 2010).

Evenness (E)

E results were calculated in Microsoft Excel using aquatic macroinvertebrate community data from all sample sites (Tables 1, 3, & 5). E = community health and productivity based on diversity; $E=H'/lnS$ (Wolf, 1996; Sponseller et al., 2001; Slye et al., 2011). E values were represented on a scale of 0 to 1, with higher values being the most significant (Wolf, 1996; Sponseller et al., 2001; Slye et al., 2011).

Richness (S)

S results were calculated in Microsoft Excel using aquatic macroinvertebrate community data from all sample sites (Tables 1, 3, & 5). S = the total number of families identified in each community (Wolf, 1996; Al-Shami et al., 2014). Higher values were the most significant (Wolf, 1996; Al-Shami et al., 2014).

Simpson's Dominance Index (D)

D results were calculated in Microsoft Excel using aquatic macroinvertebrate community data from all sample sites (Table 1, 3, & 5). D = potential for community diversity based on E and S; $D=(\sum ni(ni-1))/N(N-1)$ (Türkmen & Kazanci, 2010; Kumari & Maiti, 2020). D values

were represented on a scale of 0 to 1, with lower values being the most significant. A *D* value of one would indicate zero diversity (Türkmen & Kazanci, 2010; Kumari & Maiti, 2020).

Hilsenhoff Biotic Index (HBI)

HBI results were calculated in Microsoft Excel using aquatic macroinvertebrate community data from all sample sites and tolerance scores for each aquatic macroinvertebrate Family (Table 1, 3, & 5; McGarvey & Novotny, 2007). *HBI* = the pollution tolerance level of an aquatic macroinvertebrate community; *HBI* = ($\sum ni$xai)/N (Lillie & Schlesser, 1994; Fierro et al., 2021). *HBI* values were represented on a scale of 0 to 10, with lower numbers being the most significant (McGarvey & Novotny, 2007).

Pollution Tolerance Index (PTI)

PTI results were calculated in Microsoft Excel using aquatic macroinvertebrate community data from all sample sites and tolerance scores for each aquatic macroinvertebrate Family (Table 1, 3, & 5; Bate & Sam-Uket, 2019). *PTI* = the pollution tolerance level of an aquatic macroinvertebrate community; PTI = (Bate & Sam-Uket, 2019). PTI values were represented on a scale of 0 to 22 (Bate & Sam-Uket, 2019).

Indicator Species Analysis

Correlation analysis of sampled target aquatic macroinvertebrate families and community populations was calculated in Excel to determine indicator species (De Cáceres et al., 2010; Kubosova et al., 2010). Data were combined and compared from all sample sites for thorough analysis (Table 1, 3, 5, 7, 9, & 11; De Cáceres et al., 2010). Positive relationships with a p-value<0.05 were significant and noted (De Cáceres et al., 2010; Kubosova et al., 2010).

Canonical Correspondence Analysis (CCA)

CCA was graphed using Microsoft Excel and analyzed with PCORD-7 to uncover relationships between each sample site, the respective aquatic macroinvertebrate identifications, and aquatic habitat quality results (Tables 1-12). Aquatic macroinvertebrate families that correspond with target species families were labeled: Caenidae (*S. coushatta*), Leptohyphidae (*T. curvatus*), Perlodidae (*I. sagittata*), Hydropsychidae (*C. morsei*), Philopotamidae (*C. holzenthali*), and Dipseudopsidae (*P. harrisi*). Aquatic habitat quality vectors were labeled as Temp., DO, pH, Turbidity, and Conductivity were important parameters to understand relationships between the environment's aquatic habitat quality variables at each sample site and presence of aquatic macroinvertebrate families (Li et al., 2012; Dalu & Chauke, 2020). Greater arrow length = increased strength of relationship (Li et al., 2012; Dalu & Chauke, 2020).

CHAPTER 4

Ephemeroptera Results

Sparbarus coushatta

Sparbarus coushatta was sampled in Austin and San Jacinto Counties, June 9th, and 10th, 2023 (Fig 3 & 7). Caenidae larvae were found at all five sample sites, with greater abundance at the San Bernard River (Table 1). Caenidae larvae sampled from East Fork San Jacinto River, East Fork San Jacinto River North, and one larva from San Bernard River were dismissed from being *S. coushatta* matches because of specimen damage as well as conflicting antennae shapes (Table 1). Caenidae larvae from Little Bernard Creek, San Bernard River (Historical), and Winter's Bayou (Historical) were identified as *S. coushatta* based on exact 2.5 ratio of the first and second antennae segments, asymmetrical speckled black markings seen on dorsal body and appendages, triangular middle ocellar tubercle, crescent moon shaped tubercles on either side of cranial center, head alignment with abdomen, and patterned dorsal markings (Fig 3-6; Sun & McCafferty, 2008). The target species, *S. coushatta*, has distinctive traits that can be used to differentiate it from its two most closely related *Sparbarus* species, *S. maculatus* and *S. miccosuke*. Distinct from the absence of black femur markings in *S. maculatus*, *S. coushatta* has black femur markings that are visible dorsally (Fig 3C; Sun & McCafferty, 2008). Distinct from *S. coushatta's* 1:2.5 ratio of the first to the second antennae segments, *S. miccosuke* antennae segments exhibit a ratio of 1:2 (Fig 3B; Sun & McCafferty, 2008).

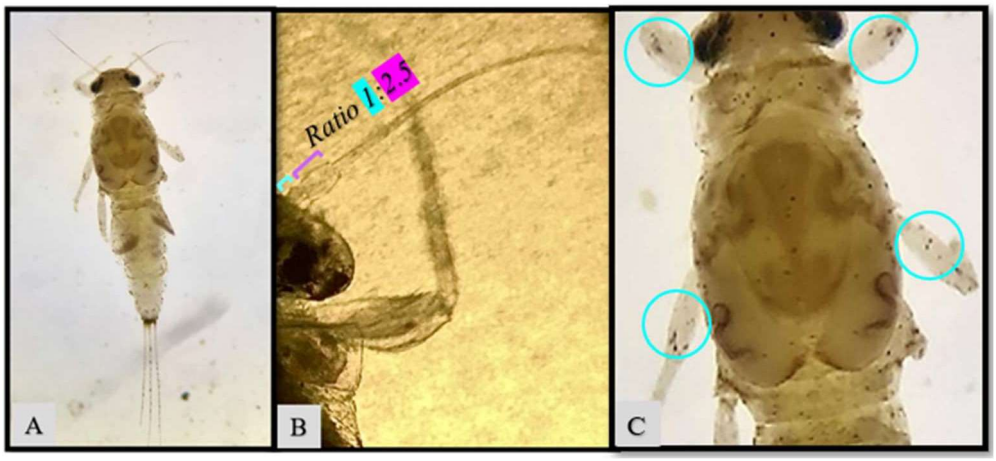

Figure 3. Caenidae *Sparbarus coushatta* larvae. A – Dorsal view. B – Dorsal view of antennae first and second segment comparison. C – Dorsal view of black femur markings. Identified as *S. coushatta* based on exact 1:2.5 ratio of the first and second antennae segments, asymmetrical speckled black markings seen on dorsal body and appendages, triangular middle ocellar tubercle, crescent moon shaped tubercles on either side of cranial center, head alignment with abdomen, and patterned dorsal markings (Sun & McCafferty, 2008). The larva shown was sampled by dip-netting the San Bernard River (Fig. 5; Table 1).

Figure 4. Little Bernard Creek. Medium high depth, murky, medium flow, rock and mud basin, riffles, heavy manmade debris.

Figure 5. San Bernard River. Shallow, slow flow and back flow, sand and rock basin, very clear, natural debris, small fish population, aquatic plants in riverbed, heavy terrestrial vegetation.

Figure 6. Winter's Bayou. Low banks, fast flow, riffles, plant debris, overhang of terrestrial plants, 5-15ft wide, sand and mud beds.

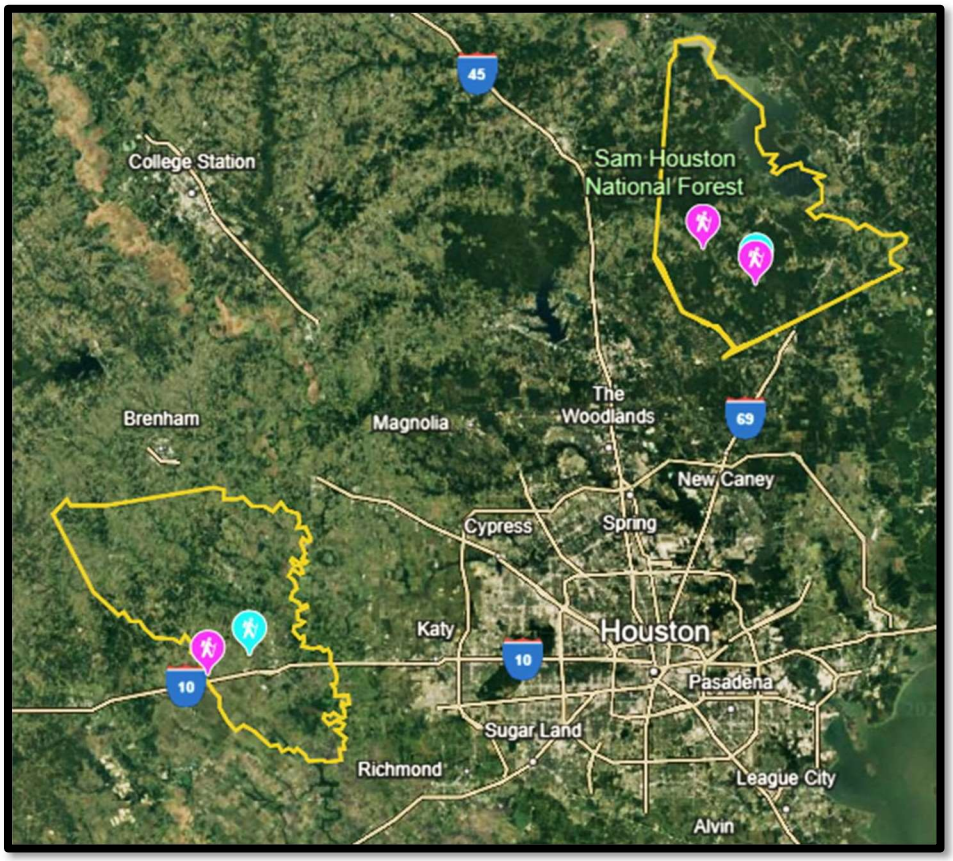

Figure 7. Austin and San Jacinto County sampling sites for *S. coushatta*: Little Bernard Creek (29.76183N/96.20522W) and San Bernard River – Historical (29.74834N/96.29726W). San Jacinto County sampling sites for *S. coushatta*: East Fork San Jacinto River – Historical (30.42509N/-95.12481W), East Fork San Jacinto River North (30.46700N/95.14707W), Winter's Bayou – Historical (30.51621N/ -95.25873W). Site map was created using Google Earth.

Table 1. Aquatic macroinvertebrate identifications from *S. coushatta* sampling sites.

Sample site	East Fork San Jacinto River (H)	East Fork San Jacinto River North	Little Bernard Creek	San Bernard River (H)	Winter's Bayou (H)
Amphipoda Gammaridae	34	0	10	9	4
Coleoptera Elmidae	17	1	7	3	0
Coleoptera Gyrinidae	3	6	0	1	2
Collembola	0	1	0	0	0
Decapoda Cambaridae	0	0	0	0	1
Diptera Ceratopogonidae	0	0	0	0	1
Diptera Chironomidae	38	12	15	99	27
Diptera Simuliidae	1	0	0	51	0
Diptera Tipulidae	3	2	0	0	0
Ephemeroptera Baetidae	1	2	5	14	10
Ephemeroptera Caenidae	1	1	4	23	1
Caenidae species IDs	1 - Non-target	1 - Non-target	3 – *S. coushatta* 1 – Non-target	22 – *S. coushatta* 1 – too damaged for ID	1 - *S. coushatta*
Ephemeroptera Heptageniidae	40	27	0	0	45
Ephemeroptera Leptohyphidae	0	0	0	8	0
Leptohyphidae species IDs				8 – *T. curvatus*	
Gastropoda Physiidae	0	0	0	0	1
Gastropoda Planorbidae	0	0	0	0	1
Odonata Aeshnidae	1	1	0	0	1
Odonata Calopterygidae	0	0	8	1	0
Odonata Gomphidae	0	0	1	1	0
Trichoptera Hydropsychidae	29	4	138	98	20
Pollution Tolerance Index	16 – Fair	13 – Fair	17 – Good	16 – Fair	15 – Fair
EPT Index	25.5%	60.0%	4.7%	12.9%	50.5%

Table 2. *S. coushatta* water quality test results. Highlighted sites indicate the presence of *S. coushatta*.

Sample site	**Temperature**	**DO**	**pH**	**Turbidity**	**Conductivity**
East Fork San Jacinto River (H)	23.5°C	6.57 mg/L	5.91	86.4 NTU	115.7 µS/cm
East Fork San Jacinto River N.	22.9°C	7.57 mg/L	5.90	79.5 NTU	64 µS/cm
Little Bernard Creek	30.7°C	6.52 mg/L	6.37	19.1 NTU	132,7 µS/cm
San Bernard River (H)	30.1°C	7.2 mg/L	6.49	19.8 NTU	126.8 µS/cm
Winter's Bayou (H)	23.6°C	7.73 mg/L	6.57	51.5 NTU	68.1 µS/cm

Tricorythodes curvatus

Tricorythodes curvatus was sampled in Montgomery County June 9th and 10th, 2023 (Fig 12). Leptohyphidae larvae were found at Caney Creek (Historical), Caney Creek South, and Peach Creek (Fig 9-11; Table 3). All Leptohyphidae larvae were identified as *T. curvatus* based on split operculate gills, black saturation of entire operculate gill, ratio of forearm length compared to width, and dorsal and appendage markings (Fig 8; Allen 1977).

Figure 8. Leptohyphidae *Tricorythodes curvatus* larvae. A – Dorsal view. B – Dorsal view of operculate gill. Identified as *T. curvatus* based on split operculate gills, black saturation of entire operculate gill, ratio of forearm length compared to width, and dorsal and appendage markings (Allen 1977). Larva shown was found in hand-picked leaf debris from historical Caney Creek (Fig. 9; Table 43).

Figure 9. Caney Creek (Historical). Old highway location. Meanders, riffles, shallow, moderate flow, sand, rock, and mud beds.

Figure 10. Caney Creek South. Riffles, medium flow, medium depth and bank heights, and few meanders.

Figure 11. Peach Creek. Riffles, medium flow, low and medium depth, heavy disturbance from human activity.

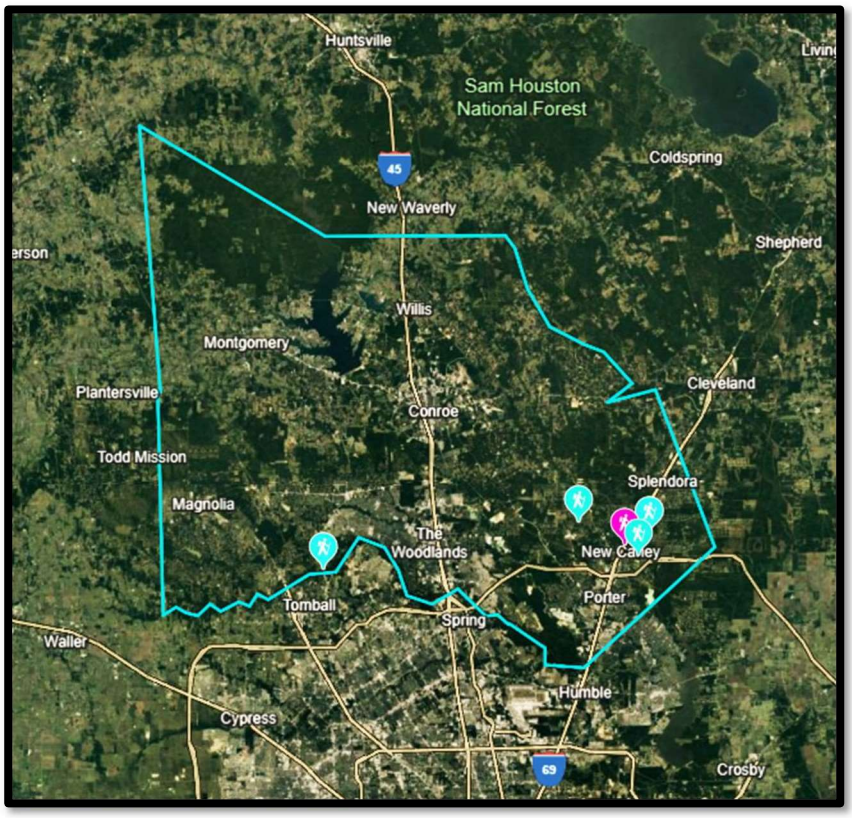

Figure 12. Montgomery County sampling sites for *T. curvatus*: Caney Creek – Historical (30.16004N/-95.20929W), Caney Creek South (30.1484N/-95.19173W), Dry Creek (30.18584N/-95.26887W), Peach Creek (30.17297N/-95.17749W), and Spring Creek (30.13453N/-95.59661W). Site map was created using Google Earth.

Table 3. Aquatic macroinvertebrate identifications from *T. curvatus* sampling sites.

Sample site	Caney Creek (H)	Caney Creek South	Dry Creek	Peach Creek	Spring Creek
Coleoptera Elmidae	13	12	13	43	1
Coleoptera Gyrinidae	0	0	0	1	6
Diptera Chironomidae	61	26	40	247	13
Diptera Simuliidae	84	73	0	44	2
Diptera Tipulidae	1	0	0	0	0
Ephemeroptera Baetidae	41	54	5	32	59
Ephemeroptera Caenidae	4	0	0	0	0
Caenidae species IDs	4 – *S. coushatta*				
Ephemeroptera Heptageniidae	27	52	29	10	0
Ephemeroptera Leptohyphidae	109	58	0	15	0
Leptohyphidae species IDs	109 – *T. curvatus*	57 – *T. curvatus* 1 – Non-target		10 – *T. curvatus* 5 – Non-target	
Hemiptera Veliidae	5	0	0	0	0
Megaloptera Corydalidae	0	7	0	0	0
Odonata Aeshnidae	1	0	3	0	0
Odonata Calopterygidae	1	1	0	0	0
Odonata Gomphidae	0	0	0	1	1
Plecoptera Perlidae	9	5	0	1	0
Trichoptera Hydropsychidae	174	123	127	333	16
Pollution Tolerance Index	19 - Good	18 - Good	11 - Fair	15 - Fair	12 - Fair
EPT Index	36.1%	41.1%	15.7%	8.0%	64.1%

Table 4. *T. curvatus* sample site water quality test results. Highlighted sites indicate presence of *T. curvatus*.

Sample site	**Temperature**	**DO**	**pH**	**Turbidity**	**Conductivity**
Caney Creek (H)	26.5°C	7.62 mg/L	6.43	51.7 NTU	14.7 µS/cm
Caney Creek South	27.1°C	7.77 mg/L	5.97	44.5 NTU	38 µS/cm
Dry Creek	24.8°C	6.22 mg/L	6.77	64.6 NTU	34.5 µS/cm
Peach Creek	27.1°C	8.35 mg/L	5.97	36.4 NTU	1.1 µS/cm
Spring Creek	25.3°C	7.92 mg/L	6.43	534.9 NTU	43.2 µS/cm

CHAPTER 5

Plecoptera Results

Isoperla sagittata

Isoperla sagittata was sampled in Newton County, February 18th, 2023 (Fig 15). Larvae of the target species' sister Family Perlidae were found at Cat Creek, Little Cow Creek (Historical), Little Cow Creek North, and McGraw Creek (Table 5). Most of the Plecoptera larvae found were easily identified as Perlidae rather than Perlodidae because of their distinct under arm gill structures. The three Perlodidae larvae found at Little Cow Creek North were identified as *I. sagittata* by their distinct upper rectangular-shaped mouthparts with a central protrusion and split between the glossae (Fig 13-14; Szcytko & Stewart, 1977).

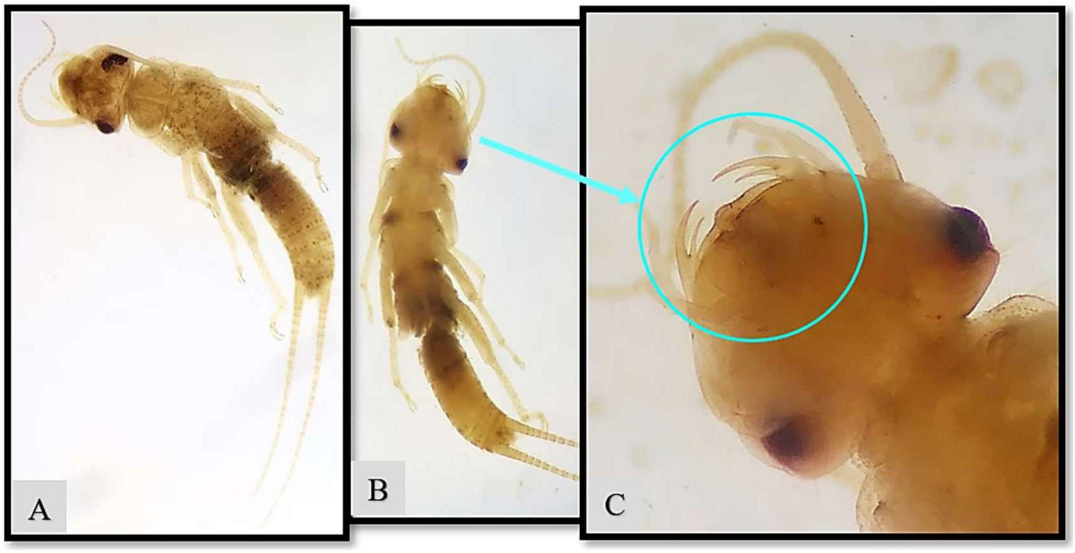

Figure 13. Perlodidae *Isoperla sagittata* larvae. A – Dorsal view. B – Ventral view. C – Ventral view of mouthparts. Specimen were identified as *I. sagittata* based on the distinctive mouthpart structures (Szcytko & Stewart, 1977). Larva shown was found with a dip-net in the sandy basin of Little Cow Creek North (Fig. 14; Table 75).

Figure 14. Little Cow Creek North. Sandy basins, fast flow, sandy banks and basin, shallow, abundant riffles, some meanders.

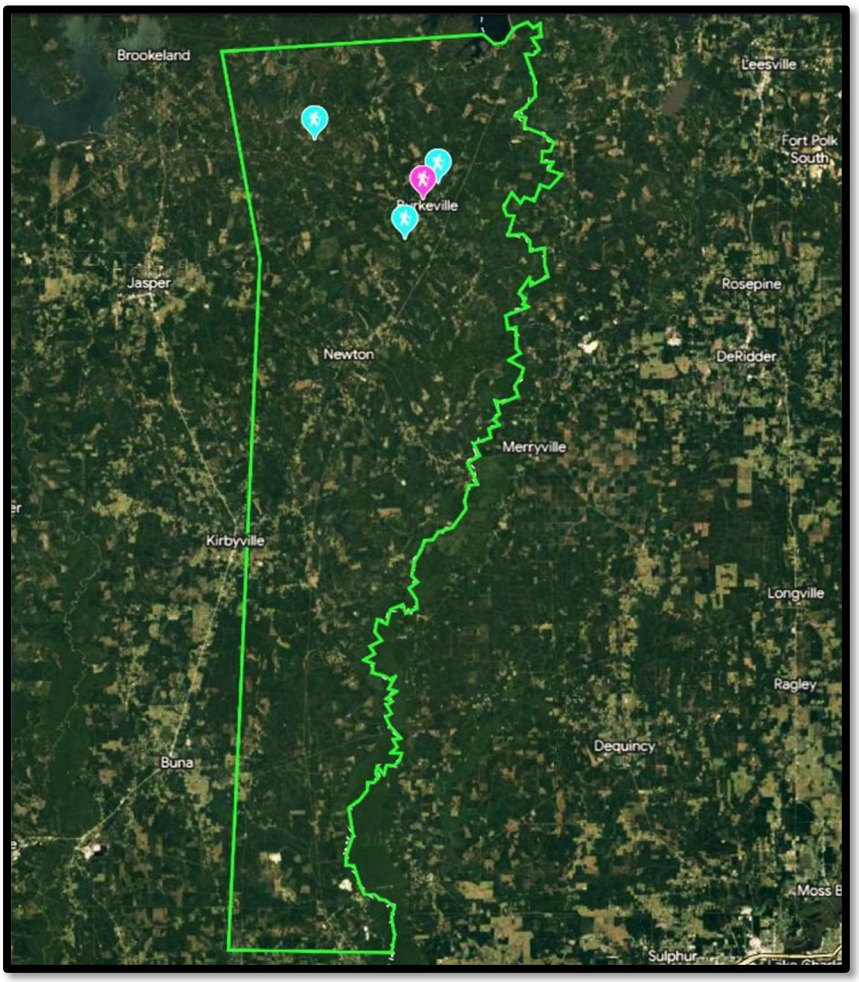

Figure 15. Newton County sampling site for *I. sagittata*. Cat Creek (30.9631078N/-93.6927571W), Little Cow Creek – Historical (30.9961206N/-93.6732178W), Little Cow Creek North* (31.0642196N/-93.8000972W), and McGraw Creek (31.03852N/-93.65527W). Site map was created using Google Earth.

Table 5. Aquatic macroinvertebrate identifications from *I. sagittata* sampling sites.

Sample site	Cat Creek	Little Cow Creek (H)	Little Cow Creek North*	McGraw Creek
Annelida Oligochaeta	0	1	2	0
Coleoptera Elmidae	1	1	0	0
Collembola	2	1	0	0
Diptera Ceratopogonidae	3	4	14	3
Diptera Chironomidae	32	17	79	12
Diptera Simuliidae	0	1	4	0
Diptera Tabanidae	0	0	2	2
Diptera Tipulidae	0	0	3	0
Ephemeroptera Baetidae	3	1	2	0
Ephemeroptera Heptageniidae	34	3	7	10
Isopoda Asellidae	1	0	0	0
Megaloptera Sialidae	0	2	1	0
Odonata Aeshnidae	0	0	2	4
Odonata Gomphidae	0	0	4	3
Plecoptera Perlidae	1	11	7	17
Plecoptera Perlodidae	0	0	4	0
Perlodidae species IDs			3 – *I. sagittata* 1 – too small to ID	
Pollution Tolerance Index	14 - Fair	15 - Fair	16 - Fair	9 - Poor
EPT Index	48.1%	40.5%	17.4%	58.7%

Table 6. *I. sagittata* water quality test results. Highlighted site indicate presence of *I. sagittata*.

Sample site	**Temperature**	**DO**	**pH**	**Turbidity**	**Conductivity**
Cat Creek	10.8°C	10.1 g/L	6.4	3.4 NTU	94 µS/cm
Little Cow Creek (H)	12°C	9.5 mg/l	6.9	0 NTU	36 µS/cm
Little Cow Creek North	8.8°C	10.3 mg/L	6.9	0 NTU	26.3 µS/cm
McGraw Creek	10.5°C	9.9 mg/L	6.9	0 NTU	26.4 µS/cm

CHAPTER 6

Trichoptera Results

Cheumatopsyche morsei, Chimarra holzenthali, and Hydroptila ouachita

Cheumatopsyche morsei, Chimarra holzenthali, and *Hydroptila ouachita* were sampled in Anderson County, June 23rd, 2023 (Fig 16). Larvae from *C. morsei's* Family Hydropsychidae were found at Box Creek, Ioni Creek (Historical), Gal Creek, Saddler Creek, and Turkey Creek (Table 170). A single larva from *C. holzenthali's* Family Philopotamidae was found at Turkey Creek (Table 170). Larvae from *H. ouachita's* Family Hydroptilidae were not found at any target sites (Table 7). The lack of presence for the *H. ouachita* Family Hydroptilidae may be because of extremely miniscule larvae sizes. Gathering live larvae of these species may require extensive in-field microscope searches. Presence of *C. morsei* = 0. Presence of *C. holzenthali* = 0. Presence of *H. ouachita* = 0.

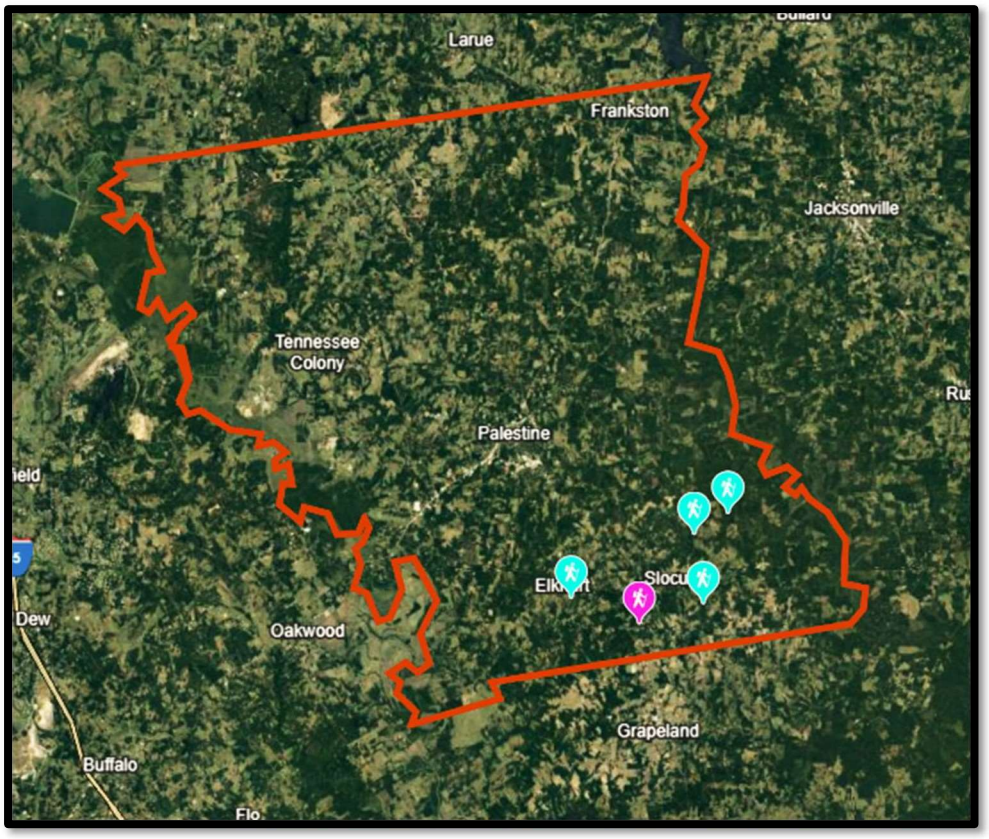

Figure 16. Anderson County sampling sites for *C. morsei, C. holzenthali*, and *H. ouachita*. Box Creek (31.61160N/-95.56954W), Gal Creek (31.68717N/-95.40325W), Ioni Creek – Historical (31.58827N/-95.49769W), Saddler Creek (31.60591N/-95.42965W), and Turkey Creek (31.66854N/-95.43918W). Site map was created using Google Earth.

Table 7. Aquatic macroinvertebrate identifications from *C. morsei, C. holzenthali,* and *H. ouachita* sampling sites.

Sample site	Box Creek	Gal Creek	Ioni Creek (H)	Saddler Creek	Turkey Creek
Amphipoda Gammaridae	0	0	0	0	41
Annelida Hirudinea	0	0	1	0	0
Annelida Oligochaeta	0	0	1	0	0
Coleoptera Dytiscidae	0	0	0	8	0
Coleoptera Elmidae	0	1	16	3	3
Coleoptera Gyrinidae	1	0	2	0	0
Coleoptera Scirtidae	4	0	0	0	0
Diptera Chironomidae	453	94	114	43	221
Diptera Simuliidae	0	0	2	0	1
Diptera Stratiomyidae	0	0	0	2	0
Diptera Tabanidae	0	0	0	1	0
Ephemeroptera Baetidae	1	5	1	2	5
Ephemeroptera Caenidae	0	0	5	0	0
Ephemeroptera Heptageniidae	1	0	4	0	2
Mecoptera Nannochoristidae	0	1	0	0	0
Megaloptera Corydalidae	8	0	3	1	6
Odonata Gomphidae	0	1	1	0	0
Plecoptera Perlidae	0	8	0	0	0
Trichoptera Hydropsychidae	191	13	152	9	36
Hydropsychidae species IDs	191-Non target	13-Non-target	152-Non-target	9-Non-target	36-Non-target
Trichoptera Philopotamidae	0	0	0	0	1
Philopotamidae species IDs					1-Non-target
Trichoptera Polycentropodidae	0	0	0	4	2
Venerida Corbiculidae	0	0	7	2	0
Pollution Tolerance Index	9 - Poor	14 - Fair	19 - Good	19 – Good	18 – Good
EPT Index	0.3%	10.7%	3.3%	34.8%	23.0%

Table 8. *C. morsei, C. holzenthali,* and *H. ouachita* sample site water quality test results.

Sample site	**Temperature**	**DO**	**pH**	**Turbidity**	**Conductivity**
Box Creek	26.0°C	7.13mg/L	6.26	12.2 NTU	78.9 µS/cm
Gal Creek	26.5°C	6.92 mg/L	6.48	19.7 NTU	109.4 µS/cm
Ioni Creek (H)	27.2°C	7.51 mg/L	6.15	13.8 NTU	110.3 µS/cm
Saddler Creek	26.0°C	7.43 mg/L	6.59	9.1 NTU	165 µS/cm
Turkey Creek	27.4°C	7.35 mg/L	6.31	4.8 NTU	84.6 µS/cm

Neotrichia mobilensis

Neotrichia mobilensis was sampled in Johnson County, June 16th, 2023 (Fig 17). Although caddisfly larvae were found at four of the five sample sites, there were no larvae found of the target species Family Hydroptilidae (Table 193). The lack of presence for the *N. mobilensis* Family Hydroptilidae may be because of the extremely miniscule larvae sizes. Gathering live larvae of these species may require extensive in-field microscope searches. Presence of *N. mobilensis* = 0.

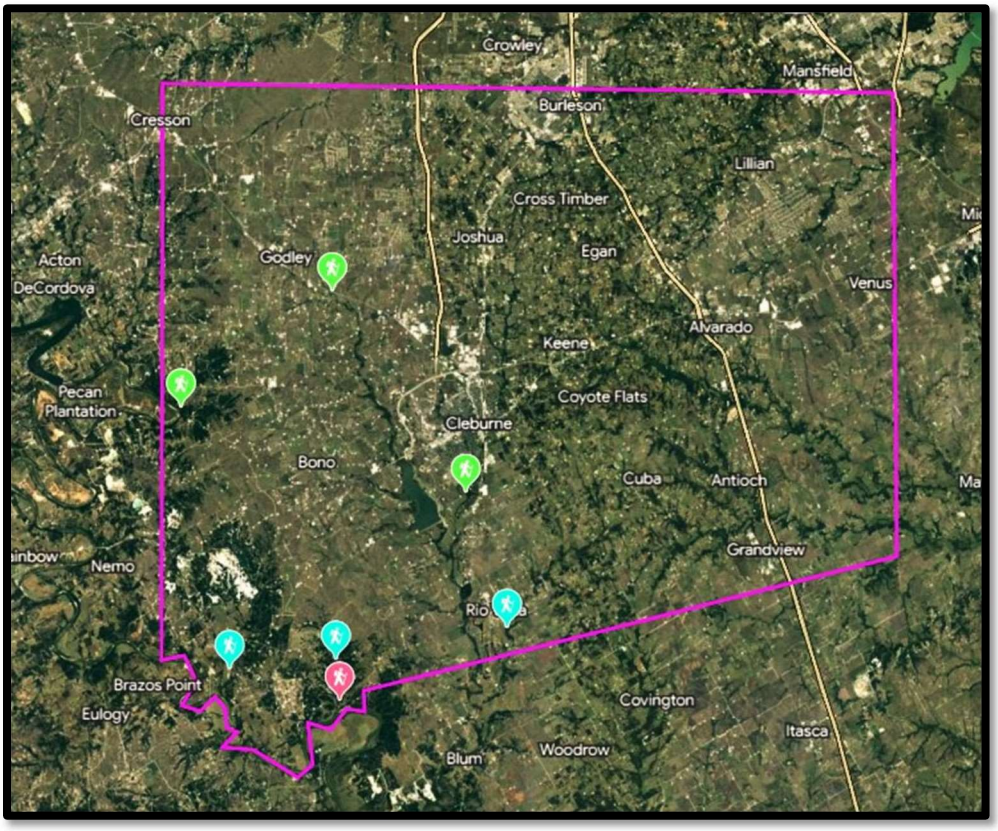

Figure 17. Johnson County sampling sites for *N. mobilensis*. Camp Creek (32.19591N/-97.56638W), Ham Creek – Historical (32.18345N/-97.49066W), McAnear Creek (32.34109N/-97.40629W), Nolan River (32.22043N/-97.39578W), and West Buffalo Creek (32.37453N/-97.39746W). Site map was created using Google Earth.

Table 9. Aquatic macroinvertebrate identifications from *N. mobilensis* sampling sites.

Sample site	Camp Creek	Ham Creek (H)	McAnear Creek	Nolan River	West Buffalo Creek
Amphipoda Gammaridae	3	0	61	0	339
Annelida Oligochaeta	0	0	1	0	0
Annelida Hirudinea	0	0	19	1	1
Coleoptera Dermestidae	1	0	1	0	0
Coleoptera Haliplidae	0	0	0	0	4
Coleoptera Hydropilidae	0	0	0	0	1
Decapoda Cambaridae	0	1	0	0	0
Diptera Chironomidae	12	3	38	1	5
Diptera Tabanidae	1	0	0	0	0
Ephemeroptera Baetidae	15	3	39	2	2
Ephemeroptera Caenidae	1	0	16	0	4
Ephemeroptera Heptageniidae	56	4	0	2	0
Ephemeroptera Leptophlebiidae	4	0	0	19	0
Gastropoda Physidae	0	0	0	0	4
Gastropoda Planorbidae	0	0	9	0	11
Hemiptera Naucoridae	0	0	0	0	1
Mecoptera Nannochoristidae	3	1	8	0	0
Megaloptera Corydalidae	0	2	0	0	0
Odonata Calopterygidae	0	0	0	0	3
Odonata Coenagrionidae	12	6	9	0	2
Trichoptera Helicopsychidae	0	0	0	16	0
Trichoptera Hydropsychidae	9	49	11	21	0
Trichoptera Odontoceridae	0	0	0	1	0
Trichoptera Philopotamidae	0	4	0	0	0
Trichoptera Polycentropodidae	1	4	0	0	0
Pollution Intolerance	17 - Good	13 - Fair	15 - Fair	10 - Poor	9 – Poor
EPT Index	70.6%	19.5%	25.9%	63.5%	1.6%

Table 10. *N. mobilensis* sample site water quality test results.

Sample site	**Temperature**	**DO**	**pH**	**Turbidity**	**Conductivity**
Camp Creek	26.5°C	7.65 mg/L	7.24	1.9 NTU	91.6 µS/cm
Ham Creek (H)	25.9°C	6.78 mg/L	7.16	0.7 NTU	78.8 µS/cm
McAnear Creek	32.7°C	9.23 mg/L	7.12	28.5 NTU	221.7 µS/cm
Nolan River	29.0°C	7.70 mg/L	7.47	44.7 NTU	102.5 µS/cm
West Buffalo Creek	30.4°C	7.24 mg/L	6.71	111.2 NTU	99.6 µS/cm

Phylocentropus harrisi

Phylocentropus harrisi was sampled in Hardin, Polk, and Tyler Counties, July 7th, and 8th, 2023 (Fig 18). Caddisfly larvae were found at five of the six sample sites (Table 11 6). Larvae from *P. harrisi's* Family Dispeudopsidae were only found at one sample site, Hickory Creek (Table 11 6). Federal protection laws prohibited direct sampling of the historical area within the Big Thicket National Forest. Because *P. harrisi* sampling sites were determined based on stream flow outside the protected historical perimeter, the chances of successfully sampling the unknown larvae were significantly reduced. Presence of *P. harrisi* = 0.

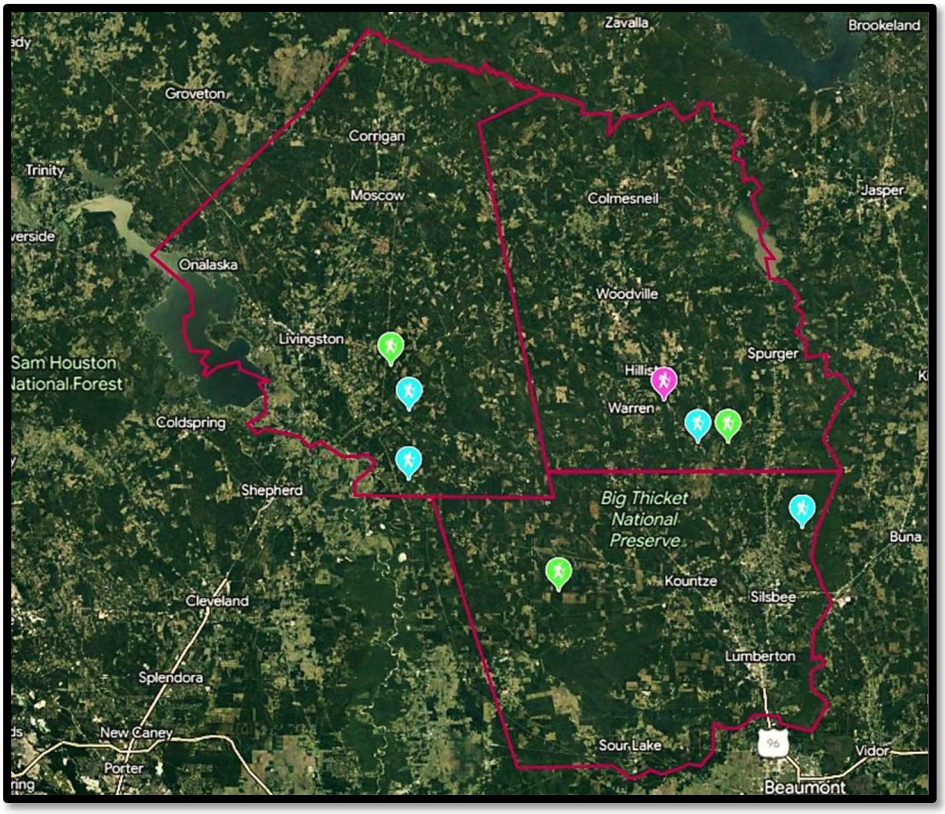

Figure 18. Hardin, Polk, Tyler County sampling sites for *P. harrisi*. Big Sandy Creek (30.71628N/-94.71667W), Big Turkey Creek (30.77593N/-94.40858W), Cypress Creek (30.35284N/-94.29460W), Hickory Creek (30.56143N/-94.39999W), Mill Creek (30.71998N/-94.69292W) and Theuvenins Creek (30.79363N/-94.37247W). Site map was created using Google Earth.

Table 11. Aquatic macroinvertebrate identifications from *P. harrisi* sampling sites.

Sample site	Big Sandy Creek	Big Turkey Creek	Cypress Creek	Hickory Creek	Mill Creek	Theuvenins Creek
Amphipoda Gammaridae	0	3	9	6	0	0
Annelida Hirudinea	2	0	1	0	0	0
Annelida Oligochaeta	0	2	3	0	2	1
Araneae Pisauridae	0	1	0	2	1	0
Coleoptera Dytiscidae	0	0	2	0	1	0
Coleoptera Elmidae	25	151	128	36	40	27
Coleoptera Gyrinidae	8	7	0	0	1	0
Coleoptera Scirtidae	0	0	1	0	0	0
Decapoda Cambaridae	2	1	00	1	1	0
Decapoda Palaemonidae	0	1	0	0	0	0
Diptera Athericidae	0	0	0	2	0	0
Diptera Chironomidae	22	60	13	12	5	29
Diptera Dixidae	0	1	0	0	0	0
Diptera Simuliidae	0	1	0	3	0	0
Diptera Tipulidae	0	0	1	0	0	0
Ephemeroptera Baetidae	9	4	0	38	11	0
Ephemeroptera Caenidae	0	0	0	0	0	4
Ephemeroptera Heptageniidae	44	30	1	43	16	8
Ephemeroptera Leptohyphidae	2	11	0	5	0	0
Ephemeroptera Leptophlebiidae	0	0	0	0	0	1
Gastropoda Physidae	3	1	0	0	0	1
Hemiptera Veliidae	1	0	0	14	0	0
Isopoda Asselidae	0	0	3	0	0	0
Mecoptera Nannochoristidae	0	0	0	1	0	0
Megaloptera Corydalidae	14	10	0	19	14	3
Megaloptera Sialidae	1	0	0	0	0	0
Odonata Aeshnidae	0	3	3	2	2	2
Odonata Calopterygidae	0	1	0	0	0	2
Odonata Coenarionidae	2	2	1	3	2	0
Odonata Gomphidae	0	3	0	0	0	0
Plecoptera Perlidae	1	3	0	14	11	1
Trichoptera Dipseudopsidae	0	0	0	14	0	0
Dipseudopsidae species IDs				14 – Non-target		
Trichoptera Hydropsychidae	27	21	1	114	10	21
Trichoptera Polycentropodidae	0	1	0	0	1	0
Pollution Tolerance	24 - Excellent	28 - Excellent	21 - Excellent	36 - Excellent	25 - Excellent	20 – Good
EPT Index	34.4%	14.5%	0.01%	34.7%	52.7%	14.0%

Table 12. *P. harrisi* sample site water quality test results.

Sample site	Temperature	DO	pH	Turbidity	Conductivity
Big Sandy Creek	25.3°C	7.42 mg/L	6.26	15.2 NTU	111.4 µS/cm
Big Turkey Creek	25.1°C	7.29 mg/L	6.03	0 NTU	188 µS/cm
Cypress Creek	29.8°C	6.89 mg/L	6.27	12.3 NTU	100.2 µS/cm
Hickory Creek	26.7°C	7.27 mg/L	6.57	15.6 NTU	60.8 µS/cm
Mill Creek	26.9°C	7.27 mg/L	6.53	17.7 NTU	49 µS/cm
Theuvenins Creek	26.5°C	7.27 mg/L	6.09	16.7 NTU	111.3 µS/cm

CHAPTER 7

Statistical Results

Fold Change

S. coushatta experienced a 22-fold increase in population abundance at its historical site of the San Bernard River, Austin County, but no fold-change at its historical site of Winter's Bayou, San Jacinto County. New *S. coushatta* abundances were found at Little Bernard Creek, Austin County, and Caney Creek, Montgomery County (*T. curvatus* historical site). *T. curvatus* experienced a 5-fold increase in population abundance at its historical site of Caney Creek, Montgomery County. New *T. curvatus* abundances were found at a southern location of its historical Caney Creek, as well as Peach Creek, Montgomery County and the San Bernard River, Austin County (*S. coushatta* historical site). *I. sagittata* experienced a 4-fold decrease in population abundance on a northern location of its historical Little Cow Creek, Newton County.

Table 13. Fold change results for identified target species: *S. coushatta, T. curvatus,* and *I. sagittata*. Sites with an identified species presence, but no historical abundance did not have a fold change calculation. Historical locations are indicated with an (H). Positive fold changes are highlighted in blue. No fold change is highlighted in yellow. Negative fold change is highlighted in red.

Species	Site	Sampled Abundance	Historical Abundance	Fold Change
S. coushatta	Little Bernard Creek	June 2023 – **3**	-	-
S. coushatta	San Bernard River (H)	June 2023 – **22**	May 1997 - **1**	*22-fold increase*
S. coushatta	Winter's Bayou (H)	June 2023 - **1**	June 1998 - **1**	*No fold change*
S. coushatta	Caney Creek	June 2023 - **5**	-	-
T. curvatus	Caney Creek (H)	June 2023 – **109**	July 1996 - **20**	*5-fold increase*
T. curvatus	Caney Creek S.	June 2023 – **57**	-	-
T. curvatus	Peach Creek	June 2023 - **10**	-	-
T. curvatus	San Bernard River	June 2023 - **8**	-	-
I. sagittata	Little Cow Creek N.	June 2023 - **3**	Feb. 1976 - **12**	*4-fold decrease*

Logistic Regression

S. coushatta species presence had a positive correlation and significant relationship with pH (p-value=0.004). *S. coushatta* species presence had a negative correlation and significant relationship with turbidity (p-value=0.033). These results indicated a higher pH and lower turbidity correlated with greater *S. coushatta* presence. *T. curvatus* species presence had a positive significant relationship with temperature (p-value=0.010). This result indicated a higher temperature correlated with greater T. curvatus presence. *S. coushatta, T. curvatus,* and *I. sagittata* species presences all had a positive correlation with dissolved oxygen (DO). *S. coushatta, T. curvatus,* and *I. sagittata* species presences all had a negative correlation with turbidity. *S. coushatta* had a positive correlation with conductivity, while *T. curvatus* and *I. sagittata* had a negative correlation with conductivity.

Table 14. Logistic regression results were calculated in Microsoft Excel for target species with identified presence in one or more sample sites: *S. coushatta, T. curvatus*, and *I. sagittata*. Shown below are the p-values and positive or negative (+/-) correlations between the aquatic habitat analyses and species presence or absence (P/A) (Tables 1-6). Significant p-values <0.05 are bolded and highlighted in blue.

Species	Temperature v. P/A	DO v. P/A	pH v. P/A	Turbidity v. P/A	Conductivity v. P/A
S. coushatta	0.192 (+)	0.900 (+)	**0.004 (+)**	**0.033 (-)**	0.597 (+)
T. curvatus	**0.010 (+)**	0.312 (+)	0.136 (-)	0.241 (-)	0.239 (-)
I. sagittata	0.129 (-)	0.317 (+)	0.667 (+)	0.667 (-)	0.603 (-)

Shannon's Diversity Index (H'), Evenness (E), Richness (S), Simpson's Dominance Index (D), Hilsenhoff Biotic Index (HBI), and Pollution Tolerance Index (PTI)

H', E, S, and *D* results show similar habitat community balances for sites with presence of *S. coushatta, T. curvatus*, and *I. sagittata*. *HBI* and *PTI* results for sites with positive presence for *S. coushatta* indicate the habitats were experiencing moderate to significant pollution. *HBI* and *PTI* results for sites with positive presence for *T. curvatus* indicate the habitats were experiencing moderate to significant pollution. *HBI* and *PTI* results for the site with positive presence of *I. sagittata* indicate the habitat was experiencing significant pollution.

Table 15. *H', E, S, D, HBI,* and *PTI* results for sampling sites of *S. coushatta, T. curvatus*, and *I. sagittata*. Results for sites with a positive species presence are shown below in bold. *HBI* results that indicate very good water quality and minimal pollution are highlighted in green. *HBI* results that indicate good water quality and moderate pollution are highlighted in blue. *HBI* results that indicate fair water quality and moderately significant pollution are highlighted in orange. *PTI* results that indicate moderate pollution are highlighted in blue. *PTI* results that indicate significant pollution are highlighted in orange. *PTI* results that indicate severe pollution are highlighted in red.

Sample Sites	H'	E	S	D	HBI	PTI
E. Fork San Jacinto River (H) *Sc*	1.78	0.771	10	0.18	5.08	16
E. Fork San Jacinto River N. *Sc*	1.56	0.771	9	0.29	4.40	13
Little Bernard Creek *Sc*	**0.965**	**0.496**	**7**	**0.55**	**4.57**	**17**
San Bernard River (H) *Sc*	**1.51**	**0.655**	**10**	**0.24**	**5.87**	**16**
Winter's Bayou (H) *Sc*	**1.66**	**0.67**	**12**	**0.25**	**4.82**	**15**
Caney Creek (H) *Tc*	**1.86**	**0.724**	**13**	**0.20**	**4.79**	**19**
Caney Creek S. *Tc*	**1.89**	**0.82**	**10**	**0.18**	**4.59**	**18**
Dry Creek *Tc*	1.21	0.675	6	0.40	4.61	11
Peach Creek *Tc*	**1.37**	**0.593**	**10**	**0.33**	**5.51**	**15**
Spring Creek *Tc*	1.21	0.624	7	0.41	5.16	12
Cat Creek *Is*	1.24	0.598	8	0.36	5.39	14
Little Cow Creek (H) *Is*	1.72	0.747	10	0.25	5.40	15
Little Cow Creek N. *Is*	**1.56**	**0.607**	**13**	**0.38**	**6.49**	**16**
McGraw Creek *Is*	1.69	0.866	7	0.20	4.10	9

Indicator Species Analysis (ISA)

ISA revealed a significant relationship between Families of two of the target species: Caenidae (*S. coushatta*) and Leptohyphidae (*T. curvatus*).

Table 16. *ISA* was calculated in Microsoft Excel using all aquatic macroinvertebrate community data (Tables 1,3,5,7,9, & 11). Results shown below are for significant correlations ($p<0.05$) with identified target species Families. A significant relationship was found between Families of two of the target species (highlighted in blue).

Family	Significant Correlation(s)	p-value
Caenidae *(S. coushatta)*	Chironomidae	0.00377
	Leptohyphidae	**0.0403**
Leptohyphidae *(T. curvatus)*	**Caenidae**	**0.0403**
	Dixidae	0.028
	Elmidae	0.0219
	Palaemonidae	0.028
Perlodidae *(I. sagittata)*	Chironomidae	0.0351

Canonical Correspondence Analysis (CCA)

The CCA vectors were important parameters to understand relationships between the environment's aquatic habitat quality variables at each sample site and presence of aquatic macroinvertebrate families (Li et al., 2012; Dalu & Chauke, 2020). The positive relationship between S. coushatta and conductivity was visualized in the plot of CCA 1 v. CCA 3 and CCA 2 v. CCA 3 (Table 13; Li et al., 2012; Olson et al., 2016; Herbst et al., 2019; Dalu & Chauke, 2020). The length of the conductivity arrow and proximity to S. coushatta's Family Caenidae indicate a strong positive relationship (Table 3; Olson et al., 2016; Herbst et al., 2019). The length of the temperature arrow in each plot and relative proximity to the target species Families Caenidae, Leptohyphidae, Dipseudopsidae, Hydropsychidae, and Philopotamidae indicated and supported a relationship between these families and the effect of temperature on their community strength (Table 20; Haidekker, 2004; Li et al., 2012; Nukazawa et al., 2018; Dalu & Chauke, 2020; Bonacina et al., 2023). The significant distance between and opposite direction of the Family Perlodidae from the other target species families indicated there was an inverse relationship between the environmental variables that affect Perlodidae in comparison to the other families (Table 20; Li et al., 2012; Nukazawa et al., 2018).

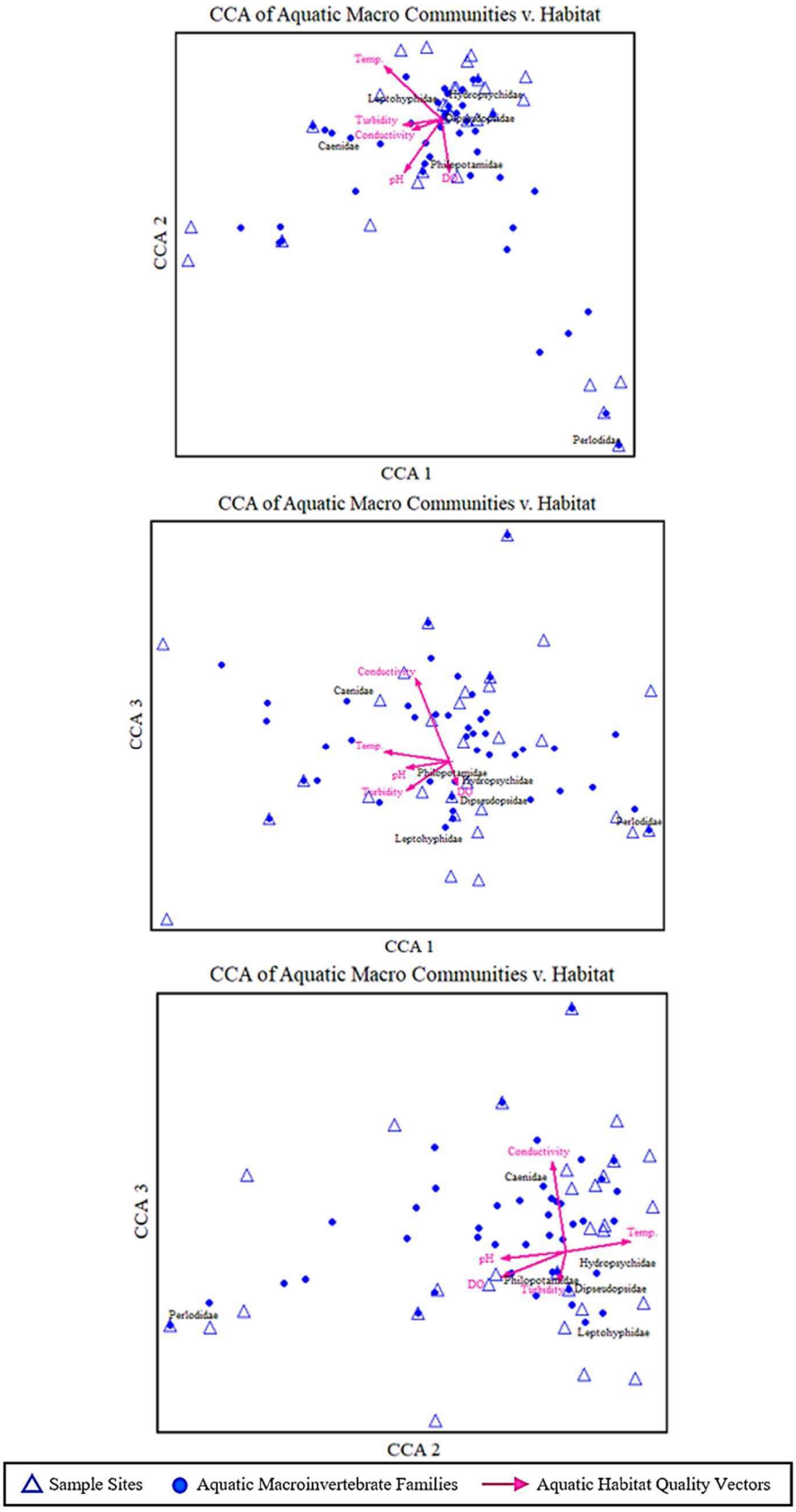

Figure 19. Canonical correspondence analysis (CCA).

Figure 19. continued… CCA was graphed using Excel and PCORD-7 to uncover relationships between each sample site (triangles), the respective aquatic macroinvertebrate identifications (dots), and aquatic habitat quality results (vectors) Aquatic macroinvertebrate families that correspond with target species families were labeled: Caenidae (S. coushatta), Leptohyphidae (T. curvatus), Perlodidae (I. sagittata), Hydropsychidae (C. morsei), Philopotamidae (C. holzenthali), and Dipseudopsidae (P. harrisi). No individuals were found from the target family of H. ouachita and N. mobilensis - Hydroptilidae. Aquatic habitat quality vectors were labeled as Temp., DO, pH, Turbidity, and Conductivity.

Chapter 8

Discussion

Three of the eight target species were identified in East Texas; *S. coushatta, T. curvatus,* and *I. sagittata*. *S. coushatta* maintained residence in its historical areas of the San Bernard River and Winter's Bayou and expanded residence to Little Bernard Creek and Caney Creek (historical location for *T. curvatus*) (Fig. 4-7; Table 1, 3, & 13). In comparison to the six *S. coushatta* larvae sampled from 1997-1998, thirty larvae were sampled in 2023 (Table 1, 3, & 13; Sun & McCafferty, 2008). *S. coushatta* larvae were found in increased overall abundance, but rarity remained (Table 1, 3, & 13; Sun & McCafferty, 2008).

T. curvatus maintained residence in its historical area of Caney Creek and expanded residence to Caney Creek South, Peach Creek, and San Bernard River (historical location for *S. coushatta*) (Fig. 4-7; Table 1, 3, & 13). In comparison to the twenty *T. curvatus* larvae sampled from East Texas in 1996, 188 larvae were sampled in 2023 (Table 1, 3, & 13; Baumgardner & Wiersema, 1999). *T. curvatus* had increased overall abundance in East Texas with sample sizes closer to its abundance found in Missouri's Meramec River (Table 1, 3, & 13; Baumgardner & Wiersema, 1999; Nichols & Sites, 1999).

I. sagittata maintained solitary residence north in its historical Little Cow Creek (Fig 8; Table 5 & 13; Szcytko & Stewart, 1977). In comparison to the twelve *I. sagittata* larvae sampled from East Texas in 1975, three larvae were sampled in 2023 (Table 5 & 13; Szcytko & Stewart, 1977). *I. sagittata* suffered increased rarity with 75% less larvae found after the 48-year sample gap (Table 5 & 13; Szcytko & Stewart, 1977).

Families were found, but no presence was confirmed for *C. morsei, C. holzenthali,* and *P. harrisi* (Table 7 & 11). No Families were found, or presence confirmed for *H. ouachita* and *N. mobilensis* (Table 7 & 9). Because there was no confirmation of the Trichoptera species' presence, their rarity may have increased.

Our target mayfly Families, Caenidae and Leptohyphidae, were found to be strong indicators (p-value=0.0403) of one another (Table 16). Evidence of this indicator species relationship was also supported in parallel species level identifications for *S. coushatta* and *T. curvatus* from Caney Creek and the San Bernard River (Table 1 & 3). Comparison of the *Shannon's Diversity Index, Evenness, Richness*, and *Simpson's Dominance Index* values for *S. coushatta, T. curvatus*, and *I. sagittata* indicated a shared similarity in community habitat diversity (Table 15). Comparison of the *Hilsenhoff Biotic Index* and *Pollution Tolerance Index* values for *S. coushatta*, *T. curvatus*, and *I. sagittata* indicated shared similarity in habitats with below average water quality and moderate to significant pollution (Table 15). These results reject our hypothesis that the eight species would be found at pristine sites with higher water quality.

A strong positive relationship was found between *S. coushatta* and conductivity (Fig. 19). High and low fluctuations of conductivity because of natural phenomena, such as rain and snow, have shown significant impacts on the health of streams and EPT species (Rezende et al., 2014). An East Texas macroinvertebrate surveys in the Big Thicket National Forest (120km east of our *S. coushatta* sampling sites) revealed the negative effects of unnatural pollutants, such as salts and chemicals, and their connection to conductivity in the sensitive stream environments (Darville & Harrel, 1980). Unnatural pollutants led to degraded water purity, increased conductivity and EPT taxa decline (Darville & Harrel, 1980). Greater conductivity in East Texas was evidence of water pollution levels with a difficult, often impossible, reversal (Darville &

Harrel, 1980; Olson et al., 2016). Our study provided current East Texas evidence that there remains a negative correlation between EPT taxa and conductivity (Fig. 19; Table 3). To increase understanding of pollutants in sensitive streams, future Texas status surveys of EPT species should sample and analyze the particulate matter of sites exhibiting high conductivity versus low conductivity (Rezende et al., 2014). If samples were taken at higher and lower regions of a stream network, the harmful and beneficial particle pathways could be tracked to determine sources of conductivity disruption (Rezende et al., 2014).

Strong significance was seen in the positive relationship between *S. coushatta* presence and pH (p=0.004) (Table 14). This relationship indicates *S. coushatta* populations prefer higher pH environments (Puckett & Cook, 2004; Buluta et al., 2010). A 2004 study, completed at Texas A &M University, tested the Family Caenidae's ability to survive various pH conditions and found greater acidity led to increased mortality (Puckett & Cook, 2004).

Strong significance was seen in the negative relationship between *S. coushatta* and turbidity (p=0.033) (Table 14). This relationship indicates *S. coushatta* populations thrive in less turbid environments (Barathy et al., 2021). Increased turbidity disrupts and decreases the available aquatic macroinvertebrate habitats (Roach & Winemiller, 2015; Barathy et al., 2021). Texas stream and river studies on turbidity's effect on the aquatic ecosystem indicated significant drops in macroinvertebrate community health and diversity (Lewis & Harrel, 1978; Davis, 1980; Davis, 1997). Turbidity blocks natural light, removes nutrition sources and if it remains, has the potential to lead to an aquatic ecosystem collapse (Davis, 1997; Roach & Winemiller, 2015). Manmade sewage drains from homes, neighborhoods, businesses, and cities are often culprits of increased turbidity (Bedient et al., 2007; Barathy et al., 2021).

Strong significance was seen in the relationship between *T. curvatus* presence and habitat temperature (p=0.010) (Table 14). A positive correlation between temperature and species presence was seen in the *S. coushatta* and *T. curvatus* populations sampled during June (Fig. 19; Table 14). A negative correlation between temperature and species presence was seen in the *I. sagittata* population sampled February (Fig. 19; Table 14). These relationships were consistent with previous findings that indicate mayfly larvae thrived in the summer months, while stonefly larvae thrived in winter months (Milner et al., 2001; Haidekker, 2004; Nukazawa et al., 2018). Wooten et al.'s (2023) stream study in central Texas examined the effects of rising temperatures on Texas streams. At the current rate of increase, Texas streams are expected to heat to a point where aquatic habitats will no longer be sustainable for sensitive macroinvertebrates because of broken temperature thresholds, increased conductivity levels, and receded water levels (Wooten et al., 2023).

One of the greatest concerns for success of EPT species status surveys has been neglect of water habitats (Harrel, 1985; Turner, 2001; Freeman et al., 2019). From the first identification of one of our target species in 1977 (*I. sagittata*) to the current 2023 sample, water quality in East Texas has declined significantly (Szcytko & Stewart, 1977; Bass & Harrel, 1981; Freeman et al., 2019). Texas freshwater studies from the past five decades exposed increased threats of pollutants by; agricultural expansions, town and business developments, and human population growth (Bass & Harrel, 1981; Harrel, 1985; Turner, 2001; Chauduri & Ale, 2014; Freeman et al., 2019; Kuwayam et al., 2020). In 2020, Kuwayan et al. compared comprehensive water quality data for all Texas river basins from 1970 to 2018. Their results showed decreased water quality expanded throughout the entire state because of human pollutants (Kuwayan et al., 2020). Efforts to restore damaged waters often prioritized human usage over natural water health and

contradicted the needs of the aquatic ecosystems (Harrel, 1985; Chauduri & Ale, 2014; Kuwayan et al., 2020). The protection of Texas waters for human usage often meant exploitation of large lakes and rivers and excluded protection of less usable streams and creeks (Harrel, 1985; Chauduri & Ale, 2014; Kuwayan et al., 2020).

There were a few major downfalls in our status survey. Search times and resources were limited. Decades had passed since the historical findings. Before we planned to search for the eight rare target species, each aquatic habitat suffered through an exponential destruction window (Freeman et al., 2019; Kuwayan et al., 2020). In our study, habitat uses varied for each sampling site. Three sites were heavily used by locals to cool off or fish in the summer heat. Five of thirty sites were homeless refuges. Seven of thirty sites were near construction areas. Twenty of thirty sites had significant manmade debris in the aquatic habitat and surrounding riparian zone. Twenty-eight of thirty sites had manmade roads, bridges, and/or buildings within three to fifteen meters of the aquatic habitat. Human encroachment has increased significantly since the eight target species were initially found and is the greatest threat to these species. The conservation status for these species should remain as Texas species of greatest conservation need (SGCN).

Conclusion

Effects of increasing human encroachment and temperatures in East Texas are projected to cause great decreases in aquatic macroinvertebrate communities and their habitats (Banner et al., 2010). The confirmed presence of three of the eight target species is of great concern to the welfare and health of these species and their freshwater habitats. To extend community preservation for aquatic macroinvertebrates, water sources throughout East Texas must be

preserved through limitations on human use and protection from construction around these habitats. To convince communities of the dire need to protect water sources, there must be more public education on the causes of aquatic habitat destruction. If the public remains disinterested and naïve to the exponential dangers of losing our freshwater sources, all freshwater habitats may be irrevocably damaged by humans.

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